Protein Interaction Domains in Clathrin-Mediated Endocytosis

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Clathrin-mediated endocytosis is the major process by which cells internalize nutrients, extracellular macromolecules, and membrane constituents to regulate such diverse processes as cell polarity, development, and motility. Over twenty proteins comprise a large protein interaction web that is pertinent to this process. This work investigates proteins that act as clathrin-associated sorting proteins (CLASPs), and their interactions with other endocytic components. Epsin 1 is shown to be a CLASP that engages components of the endocytic clathrin coat and selects for polyubiquitinated cargo. The interaction with polyubiquitin is enabled through epsin 1's UIM domains. I show that polyubiquitin is an efficient endocytic signal, which is relevant for physiological mammalian substrates such as the epithelial sodium channel (ENaC). Stonin 2 behaves as an unconventional CLASP, as it doesn't directly engage clathrin or the plasma membrane. My work uses this protein to biochemically characterize the WXXF motif and identify a privileged binding site located on the sandwich subdomain of the AP-2 α appendage. This work supports a model in which arrays of binding motifs and multiple engagement sites on the α appendage allow for an increase in binding affinity, which affects the temporal ordering of endocytic accessory protein interactions during clathrin-mediated endocytosis. These studies have defined important protein interactions that have improved our understanding of the molecular mechanism of clathrin-mediated endocytosis.

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PREFACE

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I put my mind to, and I cannot thank you enough for everything you have done for me. And finally, I would like to thank Elena for showing me what happiness truly is. I know that I can accomplish anything with you by my side. sicily

ABBREVIATIONS

AAK1	Adaptor associated kinase 1
AIDS	Acquired immunodeficiency syndrome
AMSH	Associated molecule with the SH3 domain of STAM
ANTH	AP180 N-terminal homology
AP-2	Adaptor protein 2
ARH	Autosomal recessive hypercholesterolemia
ASGPR	Asialoglycoprotein receptor
ATP	Adenosine triphosphate
BAR	BIN-amphiphysin-RVS
BSA	Bovine serum albumin
CC	Coiled coil
CLASP	Clathrin-associated sorting protein
CME	Clathrin-mediated endocytosis
CSF	Colony stimulating factor
CSP	Cysteine string protein
DNA	Deoxyribonucleic acid
EGF	Epidermal growth factor
EH	Eps15 homology

ENaC	Epithelial sodium channel
ENTH	Epsin N-terminal homology
GAK	G-associated kinase
GFP	Green fluorescent protein
GH	Growth hormone
GPCR	G protein-coupled receptor
GPI	Glycosyl-phosphatidylinositol
GRK	G protein-coupled receptor kinase
GSH	Glutathione
GST	Glutathione S-transferase
GTP	Guanosine triphosphate
HIP	Huntingtin interacting protein
HECT	Homologous to E6AP C-terminus
ITC	Isothermal titration calorimetry
IPP5c	Inositol 5-phosphatase catalytic domain
KSHV	Kaposi’s sarcoma herpesvirus
LDL	Low-density lipoprotein
Lqf	Liquid facets
LRP1	LDL receptor-related protein 1
MARCH	Membrane-associated RING-CH
MDCK	Madin-Darby canine kidney
MHC	Major histocompatibility complex
MHD	μ subunit homology domain

MIR	Modulator of immune recognition
NAK	Numb-associated kinase
NECAP	Adaptin-ear-binding coat-associated protein
ORF	Open reading frame
PCR	Polymerase chain reaction
PTB	Phosphotyrosine binding
PX	Phox
PtdIns(4,5)P_2	Phosphatidylinositol 4,5-bisphosphate
RING	Really interesting new gene
RNA	Ribonucleic acid
RNAi	RNA interference
Sac1	Supressor of actin 1
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SH3	Src homology 3
SHD	Stonin homology domain
siRNA	Small interfering RNA
SNX9	Sorting nexin 9
SOP	Sensory organ precursor
Spdo	Sanpodo
SV40	Simian Virus 40
TEN	Tensin homology
THATCH	Talin-HIP1/1R actin-tethering C-terminal homology
UBA	Ubiquitin associated

UBC	Ubiquitin conjugating enzyme E2
UbL	Ubiquitin-like
UIM	Ubiquitin interacting motif

1.0 INTRODUCTION

1.1 OVERVIEW OF ENDOCYTOSIS

1.1.1 Mechanisms of Endocytosis

The plasma membrane provides the critical interface between the intracellular and extracellular environments. This membrane is not static, but rather is a truly dynamic feature of the cell. The barrier is crucial in various physiologic functions including cell motility, regulation of growth, establishment of polarity, nutrient absorption, defense against pathogens, and ion balance [1]. One of its main functions is to regulate and coordinate the entry and exit of small and large molecules. Small molecules such as amino acids, sugars, and ions can be transported across the plasma membrane via transmembrane pumps or channels. In contrast, invagination and scission of the plasma membrane creates membrane-bound vesicles that deliver macromolecules to the cell interior in the process known as endocytosis [2]. Endocytosis is involved in all of the physiologic functions associated with the plasma membrane because it controls the protein and lipid composition at the surface, regulates signaling pathways, modulates the cell surface area, and enables the uptake of nutrients and pathogens [3].

Endocytosis is classified into two major types: phagocytosis, the uptake of large particles, and pinocytosis, the uptake of fluid and solutes [4]. The process of phagocytosis occurs in a few

specialized mammalian cell types including macrophages, monocytes, and neutrophils [5], which utilize phagocytosis to destroy large pathogens such as invading bacteria or yeast cells [6]. The process of pinocytosis occurs in all cell types [7]. The molecular mechanisms by which cells internalize fluid and solutes through pinocytosis can be subdivided into additional classes: macropinocytosis, caveolae-mediated endocytosis, clathrin-mediated endocytosis, and clathrin- and caveolae-independent endocytosis [3]. Each of these pathways utilizes distinct mechanisms and is highly regulated. They are important in the physiology of signal transduction [8], immune surveillance [9], antigen presentation [10], and homeostasis [8].

Macropinocytosis is the endocytic process that involves the internalization of sizeable areas of plasma membrane with a large volume of fluid [11]. This occurs when membrane protrusions extend and then fuse back with the plasma membrane to create irregularly shaped vesicles (>1 μm) which are called macropinosomes [12]. There is currently little known about the mechanism by which the membrane protrusions and the plasma membrane fuse.

Caveolae-mediated endocytosis has been well studied in endothelial cells, although caveolae are also found in other mammalian cell types including adipocytes, smooth muscles cells, and fibroblasts [13]. Caveolae are flask-shaped invaginations of the plasma membrane that are thought to define cholesterol- and sphingolipid-rich microdomains on the plasma membrane where many transporters and signaling molecules are located [14]. The shape of caveolae is defined by the protein caveolin [15]. These structures are involved in intracellular cholesterol trafficking and cholesterol homeostasis [16-18]. There is also evidence that caveolae have a role in the recycling of glycosyl-phosphatidylinositol (GPI) -anchored proteins, transport of glycosphingolipids, and transcytosis of serum components [19]. This type of endocytosis is important in the internalization of Simian Virus 40 (SV40) [20, 21].

The most well studied type of endocytosis is clathrin-mediated endocytosis (CME). This process occurs constitutively in most mammalian cells and will be described in detail below. The clathrin- and caveolin-independent molecular mechanisms are poorly understood and are often described according to endocytic components that are not involved. For example, in neuroendocrine cells, endocytosis occurs even when a dynamin mutant is used [22]. Dynamin is a GTPase required for budding and release in both CME and caveolae-mediated endocytosis [23, 24]. The independence of dynamin in this endocytic process suggests that this pathway is a primordial endocytic pathway; it is independent of clathrin, dynamin, and caveolae, and is simply described as such [12].

Clathrin-mediated vesicle budding is important for the internalization of receptors and extracellular ligands, recycling of plasma membrane components, and retrieval of surface proteins destined for degradation [25]. CME is critical in many aspects of development as well as in synaptic transmission, where CME provides the recycling of synaptic vesicle proteins and maintains the plasma membrane architecture at the synapse [26]. Some of the essential nutrients that CME is responsible for internalizing are iron-containing transferrin that binds to transferrin receptors, and cholesterol-rich low-density lipoprotein (LDL) particles that bind to LDL receptors [27]. In addition to these nutrients that are internalized, CME can downregulate and clear signaling receptors from the surface, thus modulating signaling cascades that are vital for cellular physiology [28].

1.1.2 Brief History of CME

Thomas Roth and Keith Porter were the first to describe the basic aspects of clathrin-coated pit formation over thirty years ago using electron microscopy [29]. This technique requires

extensive fixation that inherently sacrifices a sense for the dynamics of a process [30]. To circumvent this problem, they utilized a staged system so that the timing of cellular events could be elucidated. According to their protocol, mosquito ovaries were fixed at various durations after a blood meal to visualize the entry of yolk proteins into the oocyte [29]. Electron microscopy was then used to image the cellular events at each time point. They showed “bristle-coated pits” at the plasma membrane and observed the formation and budding of a clathrin-coated vesicle, which enabled them to propose a chronology for the cellular events of CME [29].

The study of CME was accelerated in the 1970s by several morphological studies that were performed on coated vesicles. In 1975 and 1976, two major studies conducted by Barbara Pearse described the purification of coated vesicles and the identification of clathrin as the most abundant protein in these vesicles [31, 32]. It was shown that clathrin is able to self assemble into a honeycomb-shaped lattice to form the clathrin coat at low pH [33-36]. Additional work led to the discovery of a clathrin assembly factor, a heterotetrameric protein complex found in the coated vesicles, which possessed the ability to bind clathrin and assist in the assembly of coats at physiological pH [37]. The pool of this complex that is distributed to the plasma membrane was later named adaptor protein 2 (AP-2), and it was initially suggested that the AP-2 complex is responsible for cargo sorting. It is now clear that the AP-2 complex is able to interact with short peptide sequences within cargo transmembrane proteins that define their localization inside the cell (Table 1.1) [38]. There are several sorting signals that have been identified to date [39] and these will be discussed in the following sections (Table 1.1).

Table 1.1: Internalization Signals

Signal Type	**Recognition Domain or Protein**
NPXY	PTB domain of Dab2 or ARH
YXXØ	μ2 subunit of AP-2
[DE]XXXL[LI]	μ2 and/or β2 subunits of AP-2
Ubiquitin	UIM, UBA, and UBC domains

1.1.3 Clathrin-Mediated Endocytosis Components

Clathrin-coated vesicles are assembled at the plasma membrane surface to select cargo molecules and shuttle them between the plasma membrane and underlying membrane-bound intracellular compartments. This process occurs very quickly (within minutes) and involves the deformation of the plasma membrane with the synchronous capture of cargo to create a round transport vesicle. There are four major components of CME: clathrin, heterotetrameric adaptor complexes (AP-2), transmembrane cargo receptors, and dynamin [1].

Clathrin is assembled into functional units known as triskelia, a three-legged structure that has three heavy chains and three light chains (Figure 1.1) [40]. Each heavy chain has several functional domains, a globular N-terminal region, a “knee” which segments the distal and proximal legs, and a C-terminus that allows for its trimerization [41, 42]. Three separate C-termini of individual clathrin molecule bind together to form the clathrin hub [43]. Further assembly occurs as each proximal leg of the clathrin heavy chain associate with a clathrin light chain [41, 44, 45]. The molecule is able to assemble into polyhedral lattices by packing the distal and proximal legs in an anti-parallel fashion [46]. The N-terminal region of the heavy chain has a β-propeller conformation which has binding sites for several endocytic proteins, most notably AP-2 [47]. The clathrin molecules are responsible for creating the structural part of the coat, but do not possess the ability to interact with the phospholipid membrane or cargo molecules directly. Instead, the interaction with the cargo and membrane is carried out by adaptors and accessory proteins [27, 38, 48].

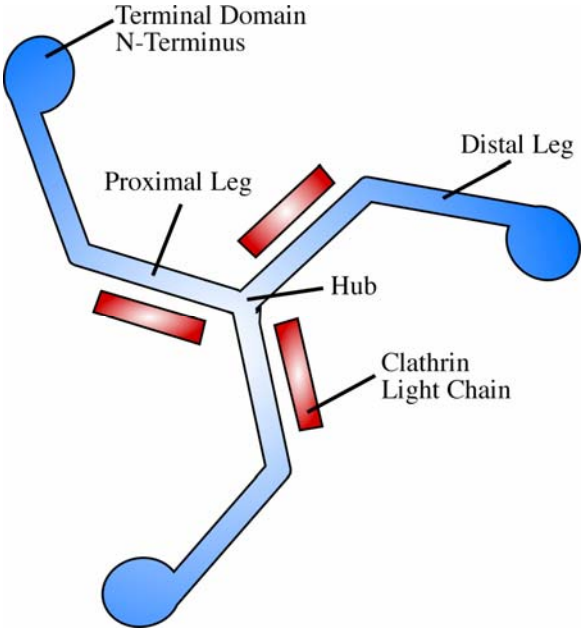

Figure 1.1: Clathrin schematic.
A schematic depiction of the clathrin triskelion modeled on the known molecular structure with the clathrin heavy chains in blue and the clathrin light chains in red. The proximal, distal, and terminal domains of the clathrin heavy chains can also be seen in this schematic.

AP-2, as introduced above, is a heterotetrameric protein complex composed of two large subunits (α and β2), a medium sized subunit (μ2), and a small subunit (σ2) (Figure 1.2) [49, 50]. The two large subunits, α and β2, are approximately 100 kDa and have an N-terminal domain (referred to as the trunk) and a globular C-terminal domain (known as the appendage) that are connected to each other via a flexible linker [49]. AP-2 has the two appendages just described and a core. The core comprises two N-terminal domains ("trunks") of the large subunits (α and β2), the μ subunit and the σ subunit [51]. The AP-2 complex has the ability to bind to clathrin via its β2 subunit [52]. In order for the transmembrane receptors to be incorporated into a newly forming clathrin coated pit, these proteins require an appropriate sorting signal. The μ2 subunit of AP-2 has the ability to bind to YXXØ sequences (where single letters designate the amino acids, with X as any amino acid, and Ø as any bulky hydrophobic amino acid) (Table 1.1) [25]. Another sorting signal is the [DE]XXXL[LI] sequence (Table 1.1) [38]. This motif acts as a strong endocytic signal, and although the precise interaction surface on the AP-2 complex has not been mapped, there is some data to support the possible engagement of the motif by a hemcomplex of α/σ2 as well as cross-linking studies showing a β2 interaction [38].

1.1.4 The Traditional Model

According to the traditional or "textbook" model of CME, the assembly of the clathrin bud is intiated by interactions between cargo and AP-2. Next, the clathrin triskelia are polymerized into a lattice, and following recruitment of additional endocytic proteins (to be discussed below), the bud invaginates. Dynamin is recruited to the necks of coated pits, where it assembles into a spiral

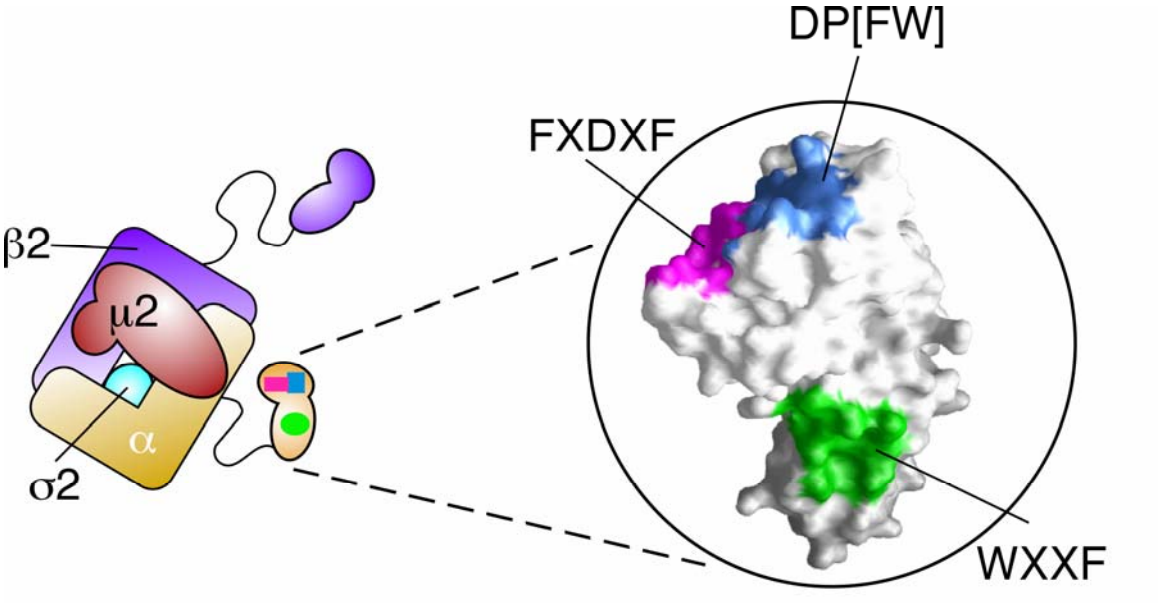

Figure 1.2: AP-2 schematic.
Schematic illustration of the AP-2 adaptor modeled on the known molecular structure. The independently-folded globular appendages project off the heterotetrameric adaptor core. The appendages are flexible due to the unstructured hinge region that connects each appendage to the core. The inset illustrates the subdomains on the α appendage with the FXDXF and DP[FW] binding sites existing on the platform subdomain and the WXXF binding site existing on the sandwich subdomain.

structure that is involved in the membrane fission and subsequent release of clathrin-coated vesicles. After successful budding has occurred, an uncoating reaction allows recycling of the components for subsequent rounds of endocytosis [11, 53].

1.1.5 Shortcomings of the Traditional Model

Although this traditional model has been generally accepted and is consistent with much data, many studies have yielded a more detailed understanding of this process. There are several important inconsistencies with the textbook model that piqued my interest to further study CME. It is now known that AP-2 does not bind cargo spontaneously; rather, a phosphorylation event is necessary for this interaction to occur [54]. The AP-2 core structures are normally in a closed state that requires a conformational change in order to engage the cargo's YXXØ motif via the μ2 subunit carboxy-terminus [51, 55]. The change in conformation occurs upon a phosphorylation event in the μ2 subunit, specifically by phosphorylation of Thr156 by adaptor-associated kinase 1 (AAK1), a member of the Ark/Prk family of kinases [54, 56, 57]. AAK1 is colocalized with clathrin-coated pits, and interestingly, the activity of this kinase is enhanced by polymerized clathrin, thus promoting cargo engagement where a clathrin lattice is assembled [58, 59]. Additional data that supports the biochemical interaction of AAK1 with AP-2 will be discussed in chapter 3.

The textbook model also does not acknowledge the role that phosphoinosides play in adaptor targeting and coat assembly. It was once believed that adaptors were recruited to the membrane via protein interactions with the cargo, but it was found that adaptors were always enriched at the plasma membrane even when cargo was not concentrated there. The structure of AP-2 also revealed the molecular mechanism by which AP-2 is able to engage membranes and

localize to specific membranes. Different cellular membranes have distinct lipid compositions; for example the plasma membrane is enriched with a specific phosphoinositide lipid, phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2) relative to other intracellular membranes. The PtdIns(4,5)P_2 lipid is concentrated at the surface by the action of PtdIns-4 and PtdIns-5 kinases, which are located at the plasma membrane [60]. AP-2 is targeted to the plasma membrane through an interaction of the α subunit N-terminus with PtdIns(4,5)P_2 by coordinating the phosphorylated inositol headgroup [61]. A second PtdIns(4,5)P_2 binding site exists on the AP-2 μ2 subunit, which allows for simultaneous lipid association and cargo binding (after phosphorylation by AAK1) by stabilizing the interaction [55, 62]. Mutation of either of these lipid-binding sites results in mislocalized AP-2 complexes [55, 61, 62]. Altering the amount of PtdIns(4,5)P_2 via overexpression or depletion systems also impacts the localization of AP-2. Increased levels of PtdIns(4,5)P_2 cause increased rates of endocytosis (with concordant increases in the amounts of AP-2 and clathrin structures at the surface) [63]. Thus, these lipids are clearly important binding partners for the subunits of AP-2 and may even provide a mechanism for regulation of recruitment to the membrane.

The importance of PtdIns(4,5)P_2 in CME is further illustrated by the studies of synaptojanin, a phosphoinositide phosphatase. Disruption of the synaptojanin gene in mice causes a phenotype that suggests a retardation of vesicle release [64-66]. PtdIns(4,5)P_2 acts as a target for the docking of clathrin coat components and its dephosphorylation is important for vesicle release. The role of synaptojanin and lipid metabolism in CME will be explored further in chapter 3.

The past several years have shown a significant increase in the identification of endocytic accessory proteins, with more than twenty recognized to date [48, 67, 68]. Biochemical and

structural studies have revealed accessory proteins that are capable of binding to the appendage domains of AP-2 via various binding motifs organized as short linear binding sequences (Figure 1.2) [48, 67, 68]. Accessory proteins contain several of these motifs embedded within unstructured regions, oftentimes linking two folded domains. The structures of the AP-2 appendages (α and β2) have been solved, and have yielded insight into how they are able to interact with these motifs [69, 70]. The appendages, despite primary sequence differences, adopt a similar structure with an N-terminal β-sandwich domain and a C-terminal "platform" domain [69, 70]. Some interacting sequences have been determined, and many of these interactions are achieved by utilizing hydrophobic residues, which have corresponding structural binding "pockets" on the appendage. Most of the peptide motifs that interact with the appendages have weak affinities, but the apparent strength of the interaction is increased by the presence of multiple repeats or motifs, as described above [71]. Examples of these AP-2 interaction motifs are the DP[FW] and FXDXF motifs (sequences are in single amino acid notation), which interact with the α-appendage through an overlapping binding site on the "platform" domain (Figure 1.2) [72]. Details about these "platform" binding sequences, as well as evidence for a novel interaction motif with a corresponding binding site on the β-sandwich domain will be discussed in chapter 3.

In addition to the AP-2 binding motifs, accessory proteins also contain binding sites for clathrin. The first identified clathrin binding motif was the clathrin box [73], a linear sequence of five amino acids, LØXØ[DE]. This clathrin box is able to bind to the N-terminus 7-bladed β-propeller domain within clathrin [52]. Another clathrin binding motif is the PWDLW amino acid sequence (termed the W-box or type II clathrin box), which also binds to the β-propeller at a distinct site [74]. In addition to the short peptide interaction motifs that bind clathrin, AP-2, and

cargo, some accessory proteins utilize a folded binding domain that can engage PtdIns(4,5)P_2 as described above [75, 76]. The traditional model does not account for these proteins satisfactorily, but it is clear that these accessory proteins use all of these functional features to cooperate with AP-2 to make buds efficiently.

Probably the largest oversimplification of the traditional model of CME is that it does not account for additional endocytic signals that are now known to exist. It is becoming more clear that different receptors utilize distinct molecular mechanisms for uptake. The most well-characterized internalization signal is YXXØ, found in the cytoplasmic tail of such proteins as the transferrin receptor and the asialoglycoprotein receptor (Table 1.1) (ASGPR) [38]. As described above, this signal sequence is recognized by the μ2 subunit of AP-2 (Table 1.1).

Even though the YXXØ motif is the best-understood internalization motif, it was not the first to be discovered. The FXNPXY sorting motif found primarily in LDL receptor family members (including LDL receptor, ApoER2, and megalin), was discovered in 1986 when the Brown and Goldstein laboratories determined that the mutation of a tyrosine to a cysteine residue in the tail of the LDL receptor prevented its internalization [77]. Additional studies confirmed that this tyrosine residue was part of the motif, NPX<u>Y</u> (later expanded to FXNPX<u>Y</u>). Historically, the YXXØ and the FXNPXY signals were grouped together as they are tyrosine based; there was even a study that suggested that AP-2 can bind FXNPXY signals in addition to YXXØ [78]. However, it is now known that the endocytic proteins Dab2 and ARH are the interacting partners for the FXNPXY motif found in these receptors [75, 79]. In summary, many recent studies have advanced our understanding of CME beyond the classic “textbook” models, and have provided additional details that have allowed detailed study of the chronology of CME.

1.2 PROTEIN-PROTEIN INTERACTIONS IN CME

1.2.1 Chronology of Clathrin Coat Assembly

The above inconsistencies and inadequacies of the textbook model have led to the development of a new, more detailed model to describe a molecular chronology of events for CME. The plasma membrane contains PtdIns(4,5)P_2 and cargo molecules. The presence of PtdIns-4 and -5 kinases generates increased local concentrations of the PtdIns(4,5)P_2 lipids at the surface, which enables the recruitment of accessory proteins and AP-2 [80]. Clathrin trimers are recruited to this site by both accessory proteins and AP-2 [71]. These trimers assemble into a lattice, which drive the recruitment of more clathrin molecules to increase the kinetics of coat formation [71, 81]. Next, the AAK1 phosphorylation event occurs within μ2 at Thr156 which allows for YXXØ cargo capture and exposes another AP-2 PtdIns(4,5)P_2 binding site that was sequestered in the unphosphorylated state [71]. Additional accessory proteins that have structural or regulatory functions are recruited, and may be involved in processes such as membrane remodeling or membrane curvature and scission to form a clathrin-coated vesicle [27]. The creation of the clathrin coat is a continuous, dynamic process, which includes an extensive network of protein-protein interactions occurring simultaneously. Late in the process, dynamin is recruited to the neck of the coated pit, and following a GTPase-dependent vesicle release, disassembly of the coat begins [1]. The enzymatic activity of the phosphoinositide phosphatase synaptojanin hydrolyzes the phosphoinositide head groups (converting PtdIns(4,5)P_2 into PtdIns), and thereby weakens the binding affinity of AP-2 and possibly other accessory proteins for the PtdIns(4,5)P_2 containing membrane [82]. Hsc70 and auxilin drive coat disassembly through an ATP-dependent reaction in which clathrin molecules are disrupted [71, 83, 84]. The uncoated

vesicle containing the cargo is able to tether and fuse with the acceptor membrane. This entire cycle is regulated by phosphorylation and dephosphorylation events on many of the coat components [71, 80, 81, 85].

1.3 ACCESORY PROTEINS AND SORTING SIGNALS IN CME

1.3.1 Early Research on Sorting Proteins

The role of accessory proteins in CME has only recently become appreciated. Some of these proteins have the ability to recognize protein cargo, or even a family of cargo [48]. One of the first accessory proteins studied was β-arrestin, but it was not appreciated as a sorting adaptor because it was initially studied with respect to G protein-coupled receptor (GPCR) signal termination, as opposed to protein and membrane trafficking. This is because visual arrestin terminates rhodopsin signaling without removal of the GPCR from the membrane. Currently, there are more than 1000 GPCRs that have been identified in the human genome, designating them as the most abundant pathway by which signals from the outside of the cell are transduced to the cell interior [86]. Many signaling receptors are rapidly internalized by CME when stimulated by their ligand to attenuate the signal. There is evidence that suggests that this pathway utilizes distinct components from the constitutive internalization pathway [87-89]. For example, the internalization of GPCRs does not rely on an amino acid sequence motif; rather, the signal that directs the endocytosis of these receptors is a phosphorylation event of the GPCR by a G protein-coupled receptor kinase (GRK) [87, 88]. The phosphorylated GPCR cytoplasmic tail is

recognized by soluble proteins, namely the β-arrestins, that link the phosphorylated GPCR to the clathrin endocytic machinery [90, 91].

The β-arrestins are responsible for controlling the magnitude and the extent of the GPCR signaling by two mechanisms. First, the β-arrestins bind to the phosphorylated cytoplasmic tail of a ligand-activated receptor and inhibit the receptor from interacting with its cognate G-protein, thereby preventing additional signaling [90, 91]. Another mechanism by which the signal transduction is attenuated is by sorting of the GPCR into a clathrin-coated pit destined for endocytosis [87, 88]. β-arrestin has a C-terminal folded domain that is able to bind to PtdIns(4,5)P_2, and an unstructured region housing a clathrin box motif able to interact with clathrin, and an AP-2 β2 appendage binding sequence [87, 88]. Upon binding of β-arrestins to activated GPCRs, a conformational change occurs that releases the disordered C-terminus from a sequestered state to allow its interaction with clathrin and AP-2, effectively targeting the GPCR-β-arrestin complex to a coated pit [87, 88].

In a mechanistically analogous manner, Dab2 and ARH are clathrin accessory proteins that promote the endocytosis of LDL receptor family members [75, 79]. These two proteins are well studied because of a hypercholesterolemia phenotype associated with LDL internalization defects. Dab2 and ARH possess an N-terminal phosphotyrosine binding (PTB) domain capable of interacting with PtdIns(4,5)P_2 as well as FXNPXY peptides (preferentially to non-phosphorylated sequences) [75, 79]. The FXNPXY motif is embedded in the cytoplasmic domain of LDL receptor family members [75, 79]. Similar to β-arrestin, Dab2 and ARH possess the ability to interact with clathrin as well as the AP-2 appendage [75, 79]. These proteins (β-arrestin, Dab2, and ARH) are the founding members of a growing family of proteins known as clathrin associated sorting proteins (CLASPs) (Figure 1.3) [27]. Thus, members of this family

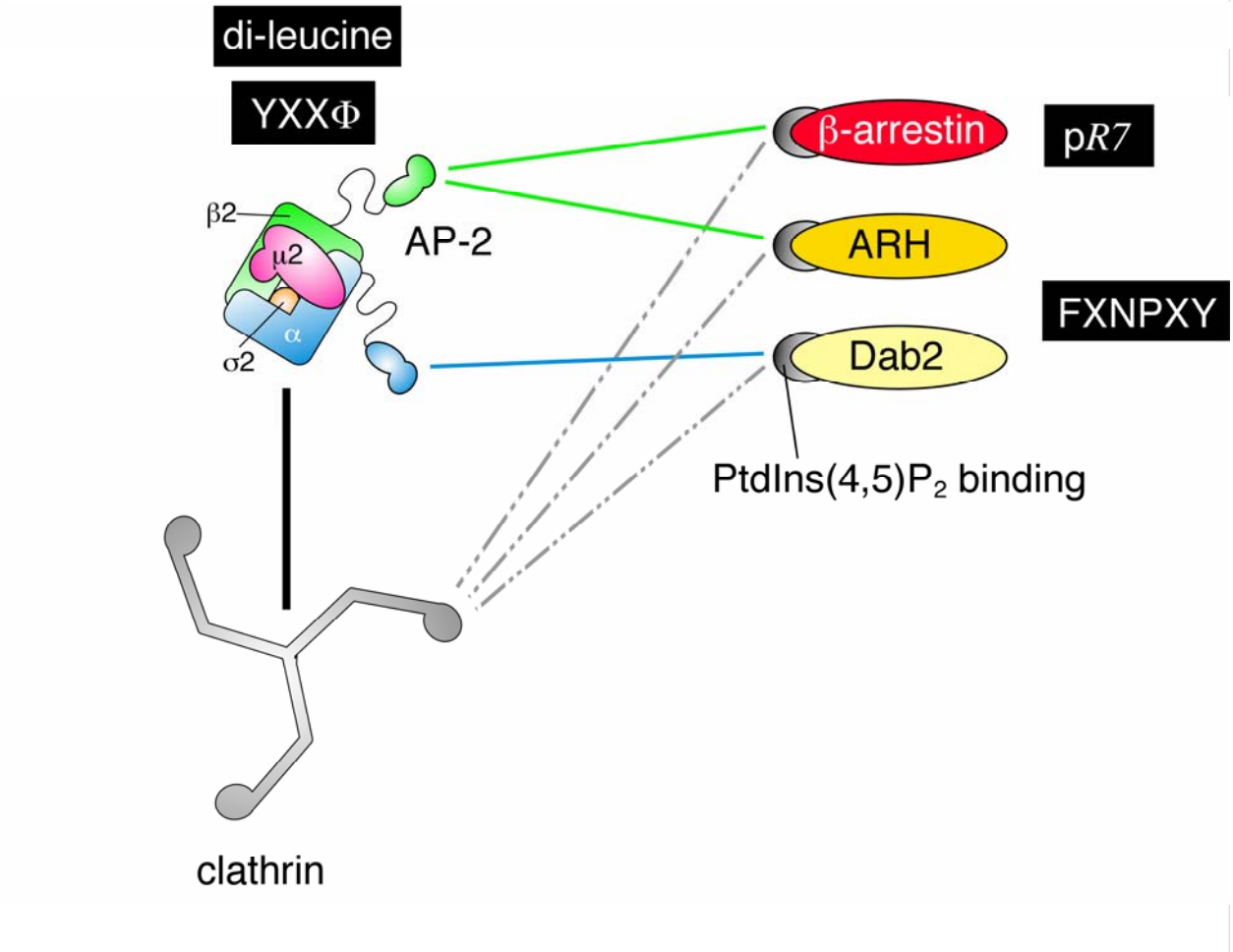

Figure 1.3: Founding members of the CLASP family of proteins.
Interactions between AP-2, clathrin and the founding members of the CLASP family are denoted by the lines. A PtdIns(4,5)P_2 interaction is indicated by the shaded oval. Known cargo sorted by these CLASPs is indicated in the black boxes.

possess four functional attributes: the ability to bind the membrane (specifically PtdIns(4,5)P_2), clathrin, AP-2 (through the appendage domains), and cargo.

1.3.2 CLASPs

The data supporting a role for dedicated sorting proteins in CME is now incontrovertible. Much of the traditional model relied upon AP-2 acting as the sole protein responsible for sorting cargo. If this were true, then depletion or knocking-out AP-2 would have a devastating effect. It turns out that deletion mutants of AP-2 in *S. cerevisiae* have no effect on CME or any other pathway [92]. Even though yeast are viable when AP-2 is deleted, when deletion mutants and temperature-sensitive alleles of a homolog of another CLASP, epsin, are utilized, these were shown to be critical for CME [93]. Although yeast can survive independently of AP-2, transgenic experiments show that multicellular organisms such as *D. melanogaster*, *C. elegans*, and *M. musculus* require AP-2 to survive [27, 94]. In support of the CLASP family of proteins, siRNA-mediated silencing of AP-2 in cultured cells caused the transferrin receptor (containing a YXXØ motif) to remain at the surface while internalization of the epidermal growth factor (EGF) receptor and LDL receptor (which contain NPXY motifs) was unaffected [95]. In addition to silencing AP-2, clathrin has also been silenced, which strongly decreases the internalization of all of the above cargo [95-97]. These data suggest the importance of other proteins beyond the traditional endocytic components in CME.

1.4 UBIQUITIN AS AN ENDOCYTIC SIGNAL AND EPSIN

1.4.1 Alternate Internalization Signals

In addition to well-characterized primary sequence internalization sequences such as YXXØ and FXNPXY, there are alternate internalization motifs. The [DE]XXXL[LI] sequence is contained within internalized proteins such as CD3-γ [38], and as mentioned above, phosphorylation serves as an internalization signal for GPCRs.

One unusual membrane internalization motif involves the post translational addition of the 76 amino acid protein ubiquitin [98]. This was an unexpected role for ubiquitin, as its well-characterized function is to promote the proteasomal degradation of proteins; in fact, in 2004, the Nobel Prize in Chemistry was awarded to Ciechanover, Hershko, and Rose for the discovery of ubiquitin-mediated protein degradation. For protein degradation, multiple ubiquitin molecules are sequentially attached to a targeted protein to create polyubiquitin chains that are recognized by the 26S proteasome for cytosolic degradation [99]. A different function for ubiquitin is now being described, in which ubiquitination of integral membrane proteins directs their endosomal-based degradation in the lysosome.

The internalization of monoubiquitin tagged proteins that lack YXXØ was first described using a *S. cerevisiae* model. The binding of α-factor and **a**-factor to Ste2p and Ste3p (GPCR pheromone receptors), respectively, induced ubiquitination on their cytoplasmic tails and subsequent endocytosis [100, 101]. Yeast endocytosis defective mutants allow for the accumulation of these ubiquitinated proteins [101]. Many other yeast plasma membrane proteins and receptors have since been shown to utilize ubiquitin as an internalization signal [102].

As stated above, AP-2 in yeast is dispensable, and ubiquitin is the major signal for endocytosis, which led to my interest in whether ubiquitin is also a mammalian endocytic signal. Research using the growth hormone (GH) receptor in a cell background defective in the ubiquitin conjugation machinery showed that its endocytosis depended on this machinery [103-108]. There are also data indicating that ubiquitination regulates the internalization of several other proteins including the following receptors: EGF receptor [109], Met [110], CSF-1 [111], as well as the epithelial sodium channel (ENaC) [108], and Delta (a transmembrane Notch ligand in *Drosophila*) [112-115]. Because the ubiquitination of certain membrane proteins can drive their endocytosis, a search for the specific components that interact with the ubiquitin tag was initiated [116].

1.4.2 Epsin 1

Using a bioinformatics approach, Hofmann and Falquet searched for proteins with amino acid homology to the ubiquitin recognizing subunit of the proteasome, S5a [117]. Two of the results were the endocytic proteins epsin 1 and eps15 as alluded to above. Epsin 1 is a CLASP and contains modular domains that interact with other components of the endocytic machinery and with membrane lipids. It has an N-terminal ENTH (epsin N-terminal homology) domain, which interacts with phospholipids at the plasma membrane; a central DP[FW] region (containing eight repeats), which interacts with the α appendage of AP-2; and a C-terminal NPF region (containing three motifs), which binds to EH (eps15 homology) domains of other endocytic proteins, including eps15 (Figure 1.4) [67]. The computer search conducted by Hofman and Falquet also revealed three tandemly arranged ubiquitin interacting motifs (UIMs) between the

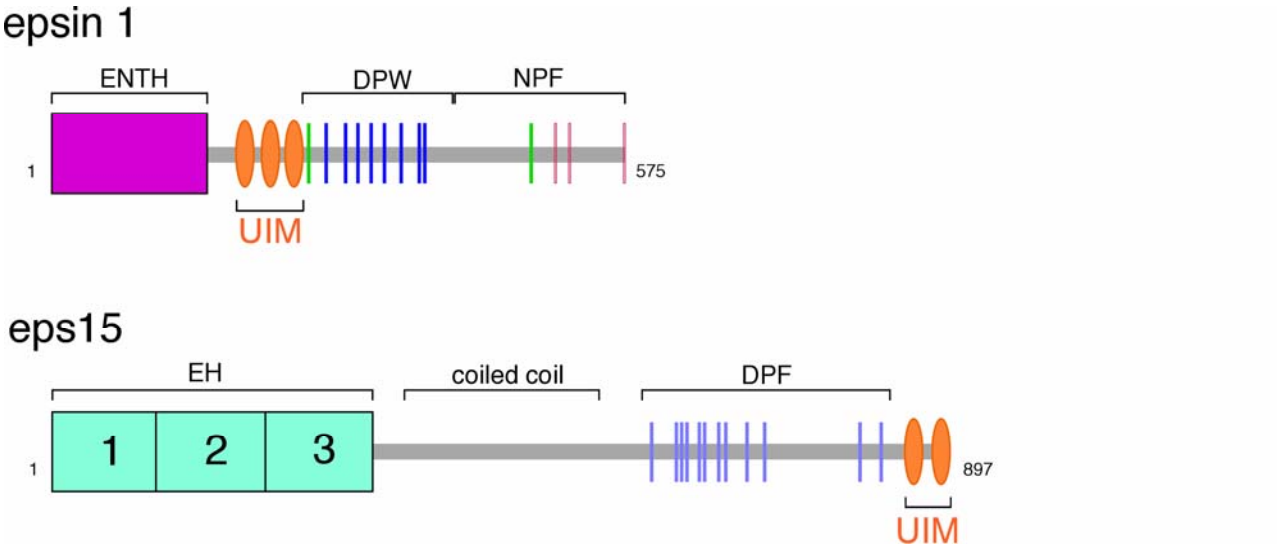

Figure 1.4: Schematic of the domain organization of epsin 1 and eps15.
Schematic illustration of the domain organization of epsin 1 and eps15 illustrating the location of the UIM sequences, AP-2-binding DP[WF] sequences (blue bars), clathrin-binding sequences (green bars), and EH domain-binding NPF triplets (pink bars). The folded ENTH (epsin N-terminal homology) domain binds physically to PtdIns(4,5)P_2 while the remainder of the protein is largely unstructured.

ENTH and DPW regions in epsin 1 [117]. Eps15, an epsin 1 interacting partner, contains two UIM sequence motifs [67]. Epsin 1 is in an optimal position to provide a cargo binding function for CME. It has the ability to bind clathrin, polymerize its assembly, and is found at steady state in coated pits at the plasma membrane in a manner similar to previously identified CLASPs such as Dab2, ARH, and AP180 [118]. In addition to immunofluorescence studies, there is ultrastructural evidence that epsin 1 is localized to a developing clathrin lattice [119]. Data from yeast studies also supports a role for epsin in ubiquitin-mediated internalization. In addition to the lethal epsin deletion phenotype described above [92, 93], precise deletion of the UIMs in the yeast epsins prevents α factor receptor uptake from the cell surface [120]. The protein-protein interactions of epsin and its potential as a CLASP for sorting ubiquitinated cargo in mammalian cells are discussed in chapter 2.

1.4.3 Role of the UIMs

When I began my work, the role of the UIMs in the context of endocytic proteins was not clear. One possibility was that the UIMs found in endocytic proteins could serve as the mammalian ubiquitin endocytic sorting machinery, facilitating the internalization of ubiquitinated proteins. One potential target molecule for the UIMs at the surface is the EGF receptor, as mentioned above. The EGF receptor family of receptor tyrosine kinases lies at the pinnacle of a complex signal transduction cascade that is known to modulate cell proliferation, survival, adhesion, migration, and differentiation [121]. On binding of ligand to the EGF receptor, the ligand receptor complex undergoes dimerization, which induces autophosphorylation, recruitment of intracellular signaling molecules, and internalization [121]. Several recent papers revealed that

the ubiquitin ligase c-Cbl is recruited to activated EGF receptor and a related receptor tyrosine kinase, the hepatocyte growth factor receptor c-Met [114, 115].

Like GPCRs, ligand-dependent downregulation of tyrosine kinase receptors such as EGF receptor and c-Met is crucial for modulating their activity. UIM-mediated sorting of ubiquitinated cargo into clathrin-coated vesicles would allow rapid entry of these proteins into the endosomal pathway for subsequent degradation in the lysosome to allow for their clearance from the plasma membrane and signal termination.

One appealing model is that a mono-ubiquitin signal initiates endocytosis while a poly-ubiquitin signal targets a protein for proteasomal degradation. Yeast apparently utilize mono-ubiquitin for cargo internalization; adding a single ubiquitin moiety in frame to the C-terminus of the H^+ ATPase Pma1p induced its endocytosis [122, 123]. Also, studies involving fully substituted arginine forms of ubiquitin (conjugation incompetent) show that, in yeast, a single ubiquitin residue is the minimal endocytic tag. Monoubiquitination has been described to cause the downregulation of receptor tyrosine kinases, immunoreceptors, and cytokine receptors in mammalian cells [124-126]. The covalent conjugation of a single ubiquitin moiety to several acceptor lysine residues in the cytoplasmic tails of these proteins (multiple monoubiquitination or multiubiquitination) was seen as the signal for endocytosis. The question that initiated much of my research is: why would the same domain in epsin and S5a recognize different substrates (monoubiquitin for epsin and polyubiquitin chains for S5a)? The S5a UIM domain was used to identify the epsin UIMs as described above, and there is a rich literature that shows that the S5a binds to tetraubiquitin or longer substrates. There is no structural explanation for the disparity in proposed ubiquitin recognition by the S5a and epsin UIMs (mono versus polyubiquitination). Chapter 2 addresses this question as well as many others and offers data to argue that

polyubiquitin chains rather than monoubiquitination serve as the signal for robust epsin 1 UIM-mediated endocytosis.

1.5 STONIN 2

Endocytosis plays a critical step in the lifecycle of a synaptic vesicle during neurotransmission. Chemical synaptic transmission requires the release of neurotransmitter via the fusion of transmitter-filled synaptic vesicles with the presynaptic plasma membrane. After fusion, synaptic vesicle proteins deposited into the plasma membrane are promptly retrieved by endocytosis and recycled to new synaptic vesicles to maintain a reserve pool of synaptic vesicles and ensure synaptic plasticity.

Defects in CME often cause a neurological phenotype due to the importance of CME in synaptic transmission. The stoned locus of *Drosophila melanogaster* was originally identified as a neurological gene in a temperature sensitive screen pioneered by David Suzuki in the 1970's [127]. When shifted to a non-permissive temperature, the stoned allele caused paralysis of the flies within a few minutes, indicating a strong neurological defect. Further evidence that the stoned gene product is involved in neuronal function stemmed from the allele-specific interaction of stoned mutants with shibire (a dynamin homolog) mutants [128]: the double mutant resulted in synthetic lethality. This genetic interaction indicated that the stoned product functions in the same pathway as shibire, which is known to be involved in endocytosis as well as synaptic vesicle recycling [129, 130].

The stoned gene gives rise to an unique bicistronic message that encodes two structurally unrelated proteins, stonedA and stonedB, from separate open reading frames (ORFs) [131].

Mutations in both ORFs produce defects in synaptic vesicle endocytosis and exocytosis, depletion of synaptic vesicles, accumulation of endocytic intermediates, and mislocalization of synaptotagmin, a synaptic vesicle protein. Both of these proteins have structural hallmarks of endocytic proteins, for example stonedA has DPF motifs while stonedB has both NPF motifs and a μ subunit homology domain (MHD) (Figure 1.5). This domain in stonedB is located in the C-terminal region and has a high identity to the μ2 subunit of AP-2 (42% identity with the μ family). Data shows that stonedB has the ability to fully restore function in mutant flies. Stoned mutations affect the function and morphology of synapses as well as neurotransmitter release [132].

Almost all stoned mutant phenotypes are similar to those described for mutants of synaptotagmin, a transmembrane protein known to regulate exocytosis and the recycling of synaptic vesicles. Like Shibire, Stoned interacts genetically with synaptotagmin to produce a lethal phenotype, and biochemically the MHD of stonedB interacts with the calcium binding C2B domain of synaptotagmin [133]. Some of this work has been performed using the human homologues of stonedB, stonin 1 and 2, as the mammalian counterpart to stonedB is highly likely to be a functional orthologue. Stonin 2 is able to bind synaptotagmin through its MHD and colocalizes with synaptotagmin at axonal vesicle clusters in cells [134, 135]. Stonin 1 only binds weakly to synaptotagmins, while stonin 2 is able to interact robustly. There are many questions to be addressed in regards to stonin 2 structure and function. It is still unclear what the function of this protein is; it seems probable that it acts as an AP-2 dependent sorting adaptor for synaptotagmin and synaptic vesicle recycling, as an atypical CLASP. The mechanism for its interaction with the endocytic machinery is unknown and will be explored in chapter 3.

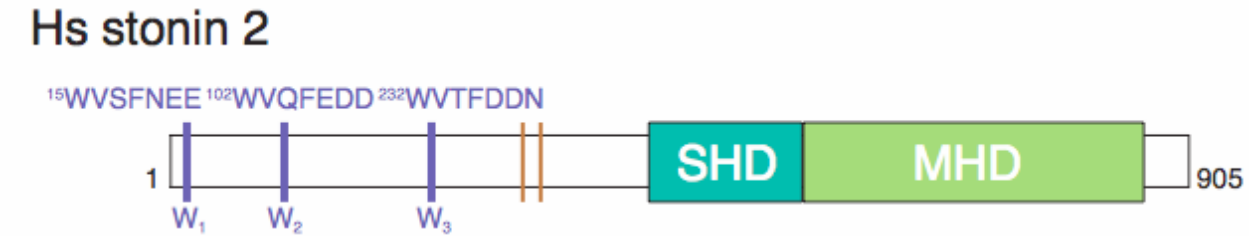

Figure 1.5: Schematic illustration of the domain organization of stonin 2.
Schematic representation of the domain organization of human (Hs) stonin 2. In this schematic are the WXXF motifs (seen in purple) as well as the stonin homology domain (SHD) and the μ homology domain (MHD).

1.6 GOALS OF THIS DISSERTATION

Clathrin-mediated endocytosis is a rapid process, but requires the presence of at least ten soluble components for successful budding of a transport vesicle. Therefore, a large network of protein-protein interactions oversees coat assembly and cargo selectivity. My dissertation research seeks to better understand a select set of endocytic interactions that contribute to clathrin function and cargo selection at the molecular level. Specifically, in chapter 2, I characterize the UIMs in epsin and demonstrate that polyubiquitin is an authentic internalization signal. Also, I identify a physiologically relevant application of the polyubiquitin modification used in the cellular internalization of ENaC. Most importantly, I show that epsin is acting as a CLASP sorting polyubiquitinated cargo proteins into a developing clathrin bud. In chapter 3, I biochemically characterize the WXXF endocytic interaction motifs present in stonin 2 and explore the biological significance of the three motif repeats. It is now clear that stonin 2 is also a CLASP, sorting different cargo and utilizing different interaction motifs for AP-2. This research has allowed the identification of a privileged binding site on AP-2 that has allowed us to better understand the chronology of protein recruitment during CME. It is critical to understand the protein-protein interactions that are important for the regulation of CME and are responsible for the uptake of extracellular nutrients, macromolecules, antigen presentation, synaptic transmission, and the downregulation or receptors.

2.0 EPSIN 1 IS A POLYUBIQUITIN-SELECTIVE CLATHRIN-ASSOCIATED SORTING PROTEIN*

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2.1 ABSTRACT

Epsin 1 engages several core components of the endocytic clathrin coat, yet the precise mode of operation of the protein remains controversial. The occurrence of tandem ubiquitin-interaction motifs (UIMs) suggests that epsin could recognize an ubiquitin internalization tag, but the association of epsin with clathrin-coat components or monoubiquitin is reported to be mutually exclusive. Here, we show that endogenous epsin 1 is clearly an integral component of clathrin coats forming at the cell surface and is essentially absent from caveolin-1-containing structures under normal conditions. The UIM region of epsin 1 associates directly with polyubiquitin chains but has extremely poor affinity for monoubiquitin. Polyubiquitin binding is retained when epsin synchronously associates with phosphoinositides, the AP-2 adaptor complex, and clathrin. The enrichment of epsin within clathrin-coated vesicles purified from different tissue sources varies and correlates with sorting of multiubiquitinated cargo and, in cultured cells, polyubiquitin, rather than nonconjugable monoubiquitin, promotes rapid internalization. As epsin

interacts with eps15, which also contains a UIM region that binds to polyubiquitin, epsin and eps15 do appear to be central components of the vertebrate poly/multiubiquitin-sorting endocytic clathrin machinery.

2.2 INTRODUCTION

Clathrin-mediated endocytosis governs not only the routine uptake of membrane and nutrient receptors, but also promotes the internalization of several ligand-stimulated receptors, ion channels, and transporters [1]. Selection of designated cargo depends upon appropriately positioned peptide internalization motifs or signals that are recognized by the protein components of the assembling clathrin lattice, retaining transmembrane cargo proteins at the bud site as invagination proceeds [38, 49]. Internalization signals were initially uncovered on analysis of a mutant low density lipoprotein (LDL) receptor that contains a single amino acid substitution within the cytosolic domain [77]. Although expressed at the cell surface, the Tyr807Cys substitution blocks the capacity of liganded LDL receptors to concentrate within clathrin-coated pits, preventing efficient LDL uptake and, ultimately, leading to pathological hypercholesterolemia [77]. Further analysis of LDL receptor endocytosis defined the cytosolic ^{802}FXNPXY internalization motif as the critical determinant recognized by the clathrin-coat machinery [136].

Several other transmembrane proteins, including the transferrin and mannose 6-phosphate receptors, use an alternate Tyr-based internalization motif [38]. Based on the consensus YXXØ, where Ø represents a residue with a bulky hydrophobic side chain, this sequence interacts directly with the μ2 subunit of the plasma-membrane specific heterotetrameric AP-2 adaptor

complex [137]. The molecular basis for this interaction has been resolved at the atomic level [138]. Mutations that disrupt the binding of YXXØ sequences to μ2, when inducibly expressed as a mutant μ2 subunit in HeLa cells, potently block transferrin internalization [139]. Indeed, the vital role that AP-2 plays in clathrin-mediated endocytosis is clearly demonstrated by RNA interference. Post-transcriptional silencing of AP-2 subunit message with siRNA oligonucleotides leads to a tenfold decrease in morphologically discernable clathrin coats at the plasma membrane [95], and severely impedes the internalization of receptors containing YXXØ-type internalization signals [95-97]. Still, clathrin-dependent internalization of several other transmembrane cargo proteins, like the LDL receptor, proceeds after extinguishing AP-2, and current evidence suggests that a family of clathrin-associated sorting proteins (CLASPs) act in conjunction with AP-2 to facilitate the rapid internalization of diverse cargo types within clathrin-coated vesicles [27, 68, 140]. Most CLASPs share four functional attributes: physical, non-competitive interactions with phosphatidylinositol 4,5-bisphosphate ($PtdIns(4,5)P_2$), clathrin, AP-2, and a designated cargo class.

Unlike nutrient cargo receptors, which internalize constitutively and repeatedly recycle back to the cell surface, many signal-transducing receptors avoid endocytic uptake until activated by ligand. For example, agonist-dependent phosphorylation of G protein-coupled receptors, like the β2 adrenergic receptor, recruits β-arrestin 1 or -2 to the receptor/membrane [141]. The β-arrestin CLASPs contain discrete interaction motifs for direct binding to both clathrin trimers [142] and the AP-2 adaptor β subunit [91, 143]. This meshes the receptor with the clathrin-coat machinery for efficient downregulation from the cell surface [141].

Another reversible signal that specifically directs transmembrane proteins at the cell surface for endocytic uptake, especially in the yeast *Saccharomyces cerevisiae*, is ubiquitin [108,

116]. In fact, both the β2 adrenergic receptor and β-arrestin are rapidly ubiquitinated upon ligand stimulation [144]. This likely increases the fidelity of receptor uptake after stimulation, but the identity of the components of the clathrin machinery that interface with ubiquitin in vertebrate cells remains controversial. We show here that epsin 1, a protein known to interact efficiently with the core endocytic components AP-2 and clathrin and with PtdIns(4,5)P_2, binds to polyubiquitin via the computationally identified ubiquitin-interaction motifs (UIM) sequence [117]. These results extend our characterization of the emerging family of cargo-specific monomeric endocytic adaptors we term CLASPs [27], and argue against epsin 1 (and its binding partner eps15) functioning to sort monoubiquitinated cargo via caveolae [145, 146].

2.3 RESULTS

The positioning of two or three tandem UIMs (Figure 2.1A and B) within alternatively-spliced isoforms of epsin 1 [117] has prompted the proposal that epsin (and the *S. cerevisiae* orthologues Ent1/2p), perhaps together with the binding partner eps15 (which also contains 2 UIMs [117]), governs the retention of monoubuiquitinated cargo molecules in assembling clathrin buds [125, 147, 148]. Indeed, *in vitro*, epsin 1 binds physically to ubiquitin (Figs. 2.2-2.4). When immobilized upon glutathione-Sepharose, neither GST alone nor residues 1-163 of epsin 1 fused to GST (GST-ENTH, Figure 2.1A) associate with ubiquitin or with polymeric ubiquitin chains (Figure 2.2, lane b and d). By contrast, residues 1-407 fused to GST (GST-ENTH+DPW) bind polyubiquitin chains robustly (lane f) and deplete the higher-order ubiquitin chains from the supernatant fraction (lane e compared to lanes a and c). Significantly, association with di- or triubiquitin conjugates is minimal in this assay. That the interaction with the Lys48-linked

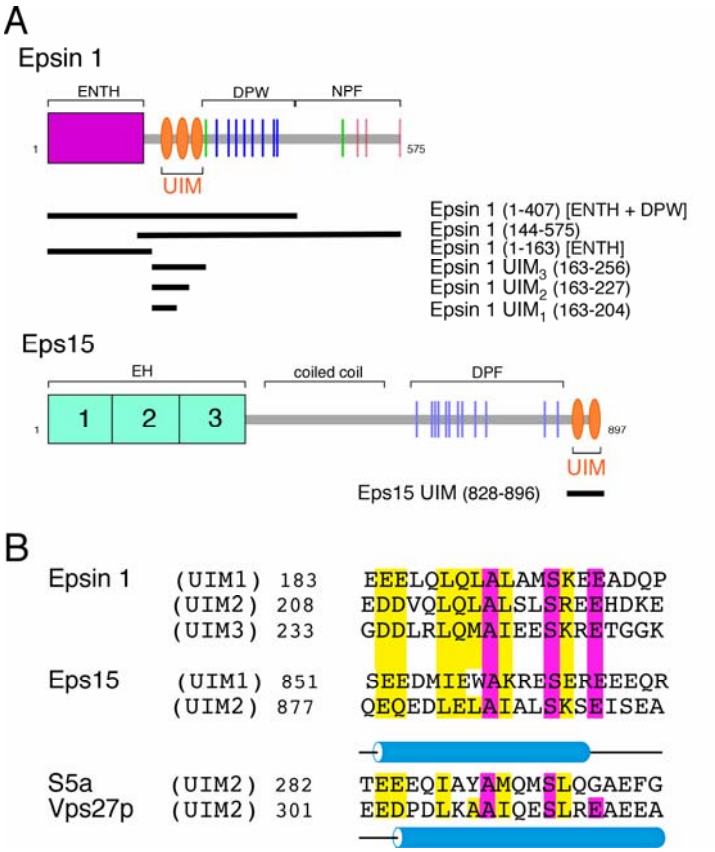

Figure 2.1: Schematic illustration of the domain organization and sequence alignment of epsin 1 and eps15. A) Schematic illustration of the domain organization of epsin 1 and eps15 illustrating the location of the UIM sequences, AP-2-binding DP[WF] sequences (blue bars), clathrin-binding sequences (red bars), and EH domain-binding NPF triplets (black bars). The folded ENTH (epsin N-terminal homology) domain binds physically to PtdIns(4,5)P_2 while the remainder of the protein is largely unstructured. The location of the different constructs used in this study are shown in relation to the intact polypeptides. B) Sequence alignment of the epsin, eps15, S5a and Vps27p UIM repeats. Invariant residues are boxed in magenta while chemically conserved residues are boxed in yellow. The location of the UIM helix, based on structural studies, in the S5a and Vps27p UIMs is indicated.

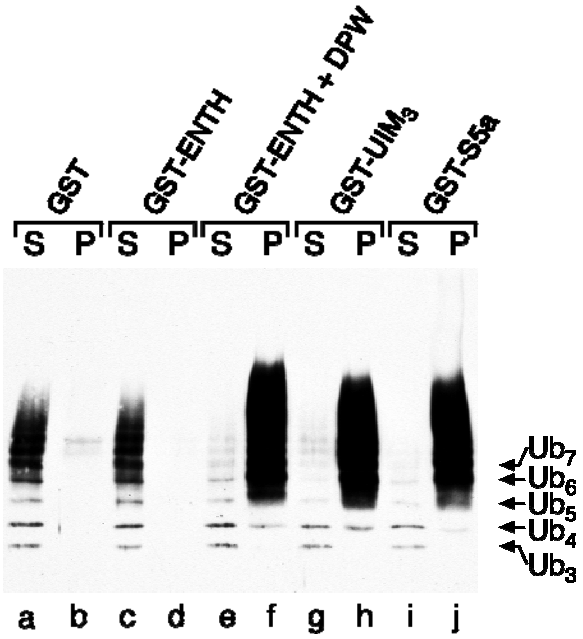

Figure 2.2: Binding of Lys48-linked polyubiquitin chains to various epsin constructs.
Binding of Lys48-linked polyubiquitin chains to 5 μg of immobilized GST or the indicated GST-fusion proteins. Approximately 10-fold more of each pellet (P) compared to the corresponding supernatant (S) fraction was analyzed on immunoblots with anti-ubiquitin antibodies.

polyubiquitin chains is due to the triple UIM (UIM_3)-containing region of epsin 1 is confirmed by a GST fusion of only epsin 1 residues 168-256 (GST-UIM_3) retaining full ubiquitin-binding capacity (lane h) [149]. Notably, the extent of UIM_3 binding to polyubiquitin chains is similar to that of the GST-S5a control protein (lane j), an authentic polyubiquitin-recognizing subunit of the 19S regulatory particle of the 26S proteasome [150, 151].

Thus, *in vitro*, the epsin UIM_3 segment has an extremely weak apparent affinity for monoubiquitin. The strong preference of the epsin UIM_3 for ubiquitin chains composed of four or more covalently-coupled monomers is apparent irrespective of whether the chains have a Lys48 or Lys63 linkage (Figure 2.3). Efficient binding to polyubiquitin depends upon multiple UIMs (Figure 2.4). Compared to the epsin GST-UIM_3, which has the three tandemly-arrayed UIMs, GST-UIM_2 and GST-UIM_1 bind negligible amounts of ubiquitin. This result could indicate that the last of the three sequentially-arrayed UIMs in epsin 1 binds ubiquitin with high apparent affinity. In fact, in S5a the two UIMs do display 5-10-fold different affinities for ubiquitin [150], as a result of the precise positioning of the invariant UIM anchor Ala and Ser side chains relative to the overall UIM α-helical element, and also due to non-conserved N-terminal residues which, in the second S5a UIM, contact ubiquitin [151]. However, when expressed alone, neither the first nor the third UIM of epsin 1 binds ubiquitinated proteins efficiently [152], supporting our conclusion that polyubiquitin binding requires multiple UIMs.

In our pulldown assays, a hundredfold excess of monoubiquitin does not alter the binding of >tetraubiquitin chains to the epsin 1 UIM_3 region fused to GST (Figure 2.5) and, similarly, a large excess of monoubiquitin does not impede the association of the immobilized epsin UIM_3 with uncharacterized polyubiquitinated proteins within a HeLa cell extract (Figure 2.6). These results are similar to the ~300-fold higher affinity of the UBA domain for tetraubiquitin chains

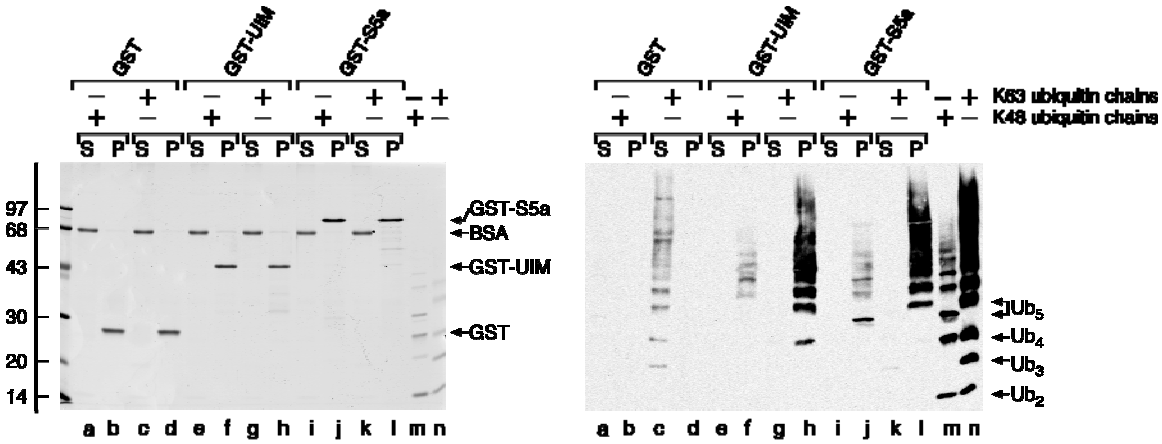

Figure 2.3: Comparison of Lys48- or Lys63-linked polyubiquitin chains.
Binding of Lys48- or Lys63-linked polyubiquitin chains to 5 μg of GST, GST-epsin UIM or GST-S5a. Approximately 10-fold more of each pellet (P) fraction compared to the corresponding supernatant (S) fraction was resolved by SDS-PAGE and stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin antibodies. Note that chain linkage affects reactivity with the anti-ubiquitin antibodies; the higher-order Lys63-linked chains react considerably better (right panel), despite roughly equivalent amount of protein (left panel).

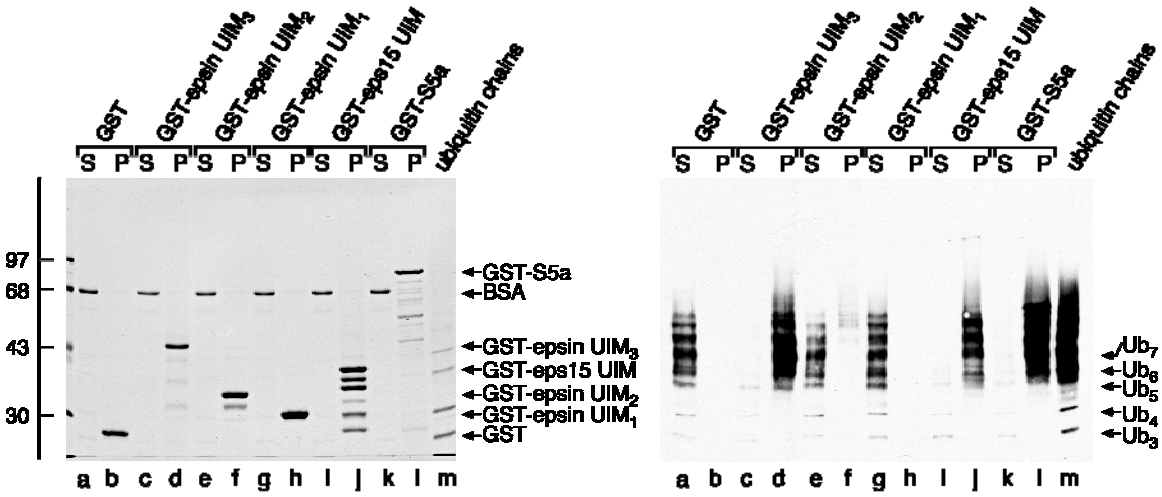

Figure 2.4: Epsin UIM truncations.

Binding of Lys48-linked polyubiquitin chains to 5 μg of GST, GST-epsin UIM_3, GST-epsin UIM_2, GST-epsin UIM_1, GST-eps15 UIM, or GST-S5a. Approximately 10-fold more of each pellet (P) fraction compared to the corresponding supernatant (S) fraction was resolved by SDS-PAGE and stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin antibodies. The selective loss of the largest ubiquitin polymers from the supernatant fraction after incubation with GST-epsin UIM_2 (lane e compared to lanes a and g) indicates only weak association with two epsin UIMs, and the limited recovery of these ubiquitin chains observed in the pellet (lane f) suggests dissociation and loss during the washing steps. Note, however, that both the eps15 and S5a only have two known UIM repeats.

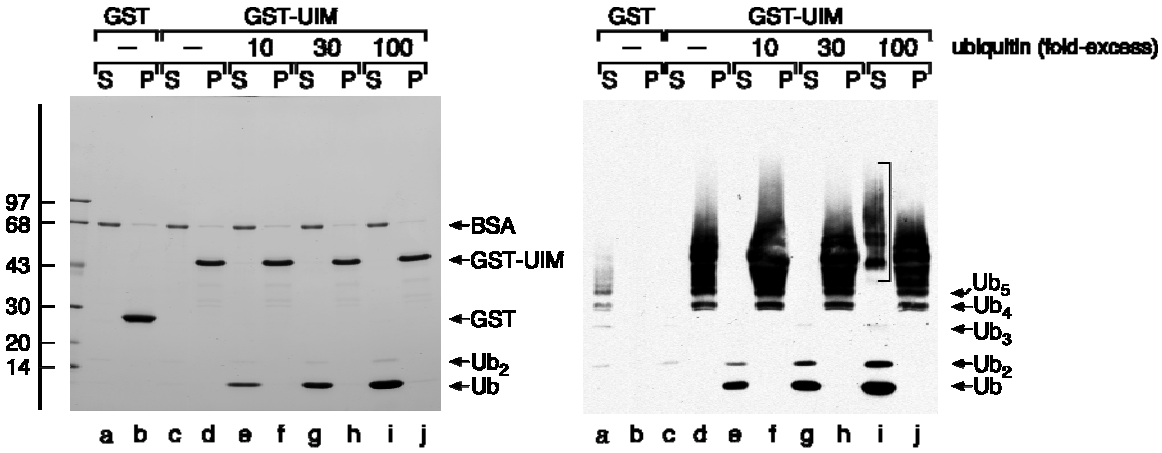

Figure 2.5: Effect of excess monoubiquitin on the binding of polyubiquitin chains to immobilized GST-UIM. Effect of 10–100-fold molar excess of monoubiquitin on the binding of polyubiquitin chains to 5 μg immobilized GST or GST-epsin 1 UIM_3. Approximately 10-fold more of each pellet (P) compared to the corresponding supernatant (S) fraction was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin antibodies. Note that at high concentrations of added monoubiquitin (lane i) nonspecific high-molecular weight aggregates are detected (bracket).

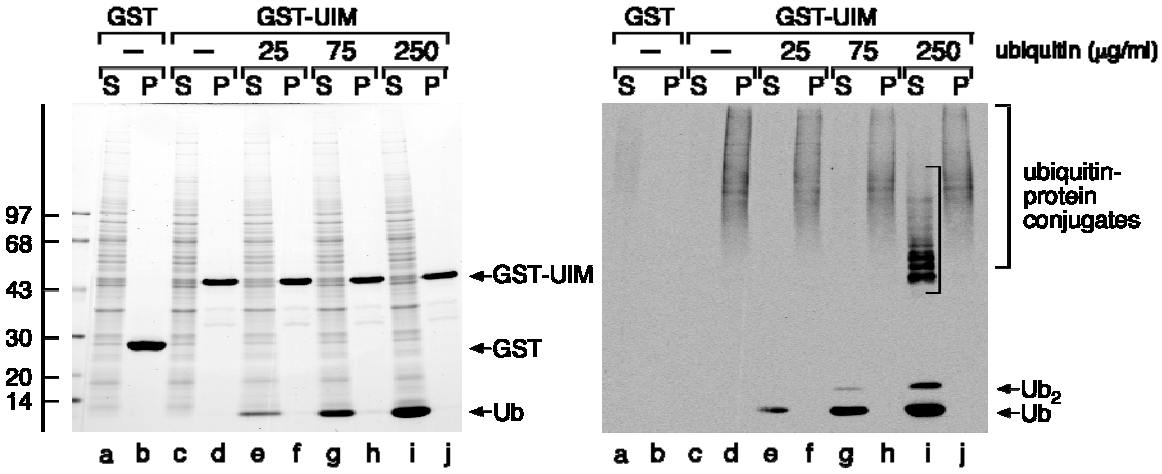

Figure 2.6: Effect of excess monoubiquitin on the association of HeLa lysate polyubiquitin proteins.
Effect of excess monoubiquitin on the association of uncharacterized polyubiquitinated proteins present within HeLa cell lysate with 5 μg of immobilized GST or GST-epsin 1 UIM_3. Approximately 10-fold more of each pellet (P) fraction compared to the corresponding supernatant (S) fraction was resolved by SDS-PAGE and stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin antibodies. Again note that at high concentrations of added monoubiquitin (lane i) nonspecific high-molecular weight aggregates are detected (bracket). GST-S5a affinity isolates the same set of proteins from the HeLa cell lysate and essentially identical results are obtained using the anti-polyubiquitin mAb FK2.

over monoubiquitin [153]. Importantly, the marked preference of the epsin 1 UIM_3 domain for polyubiquitin does not change when the protein associates with a PtdIns(4,5)P_2-containing bilayer. Bound to a liposome surface, the epsin 1 ENTH domain fails to associate with either mono- or polyubiquitin (Figure 2.7, lane d and f), but the epsin 1 ENTH+DPW segment (residues 1-407, which encompasses the UIM_3 segment; Figure 2.1A) selectively binds higher-order Lys48-linked polyubiquitin chains (lane l) over mono- or diubiquitin (lane j and l). This result is significant because His68, located on the periphery of the common hydrophobic interaction surface upon the ubiquitin molecule, is reported to interfere with UIM engagement [154, 155] and it is suggested that protonation, possibly promoted by a phosphoinositide-created low pH environment, might regulate UIM engagement [155].

It has recently been proposed that epsin 1 binding to ubiquitin or AP-2 and clathrin are mutually exclusive events, and that epsin 1 promotes the internalization of ubiquitinated cargo molecules in a clathrin-independent manner [145, 146]. Yet, upon liposomes, epsin 1 (residues 1-407) associated with both AP-2 and clathrin is still able to interact with polyubiquitin, while AP-2 and clathrin do not (Figure 2.8, lane h compared to lanes b and f). A substantial fraction of the bound polyubiquitin chains sediments following solubilization of the liposome membrane with Triton X-100 (lane j), indicative of incorporation into extensive polyhedral clathrin assemblies. Obviously, epsin 1 (1-407) alone is also able to bind clathrin and conjugated ubiquitin oligomers when associated with the liposome surface (lane d). Although we cannot be certain that each epsin molecule is bound simultaneously to both AP-2 and clathrin under these conditions, the relative stoichiometries (lanes d and j) suggest numerous contacts are possible and, after Triton X-100 solubilization, the epsin must be bound to either AP-2 or clathrin to sediment with the coat assemblage.

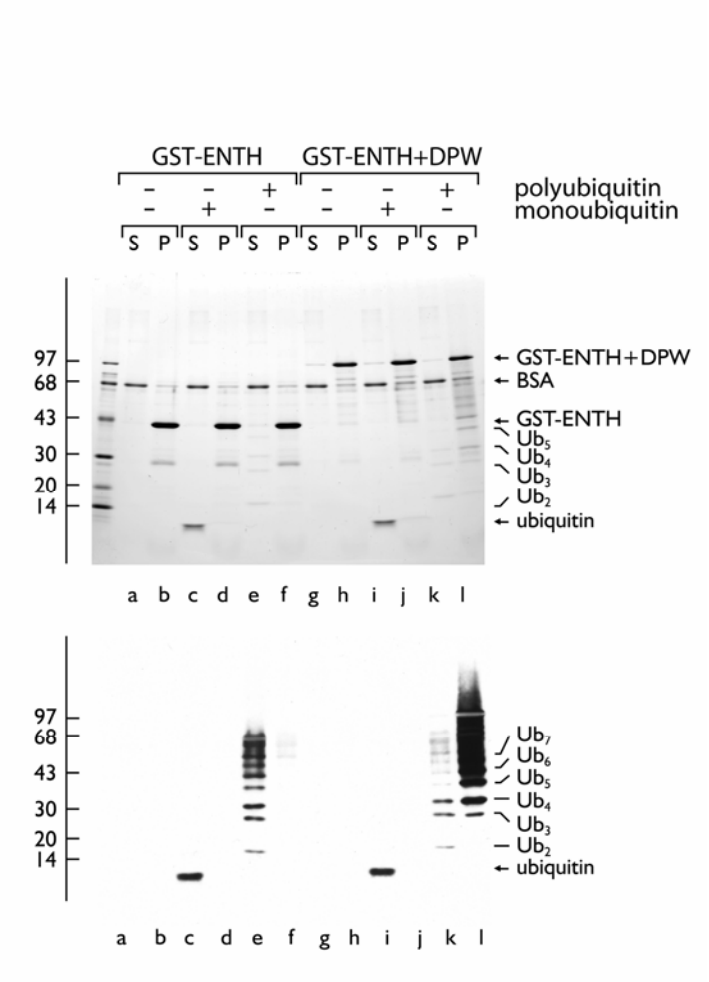

Figure 2.7: Productive association of epsin 1 with polyubiquitin chains in the context of phosphoinositide containing liposomes.

PtdIns(4,5)P_2-containing liposomes were preincubated with 20 μg of either epsin 1 ENTH or ENTH+DPW domain and, after centrifugation and liposome resuspension, 12 μg of ubiquitin or 6 μg of Lys48-linked ubiquitin chains added as indicated. Following centrifugation, 8-fold more of each pellet (P) compared to each supernatant (S) fraction was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin antibodies.

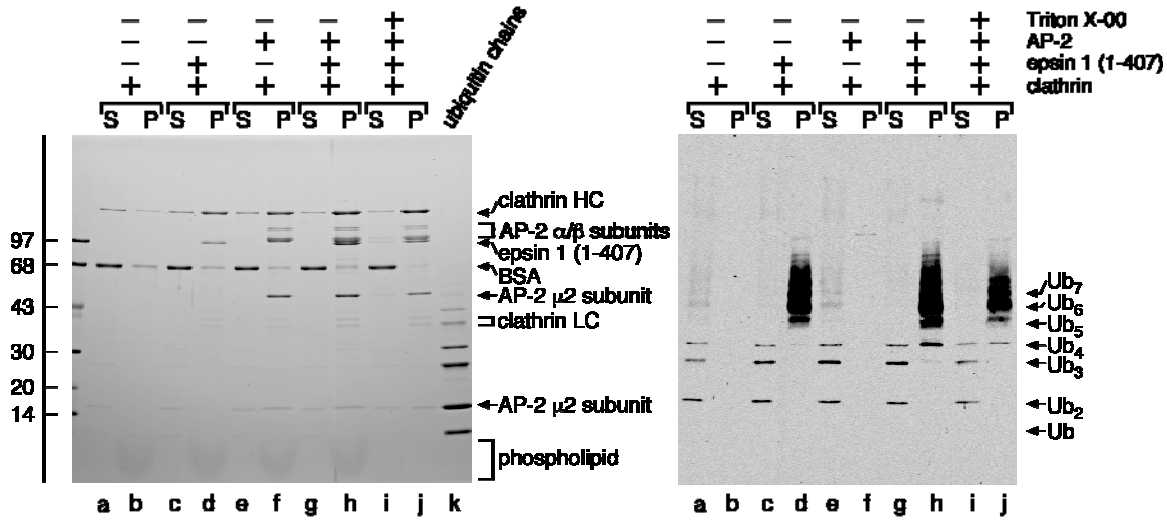

Figure 2.8: Productive association of epsin 1 with polyubiquitin chains in the presence of AP-2, clathrin, and phosphoinositide containing liposomes.

PtdIns(4,5)P_2-containing liposomes were incubated with 5 μg epsin 1 (1-407) and 8 μg AP-2 and then, after centrifugation and liposome resuspension, with 5 μg clathrin and 6 μg Lys48-linked ubiquitin chains as indicated. After the second-stage incubation, Triton X-100 was added to one tube to 1% and, following centrifugation, approximately 8-fold more of each pellet (P) compared to each supernatant (S) fraction was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin antibodies. Note that in the absence of Triton, faint staining of acidic phospholipids can be seen at the bottom of the stained gel.

Because epsin 1 contains two clathrin-binding sites that flank the eight tandemly arrayed, AP-2-binding DPW motifs (Figure 2.1A) the protein binds physically to clathrin [118, 156] and alone can assemble clathrin trimers into polyhedral cages [157]. When bound to assembled clathrin, epsin 1 (144-575) promotes the cosedimentation of polyubiquitin, but not monoubiquitin, with the cages (Figure 2.9, lane d). Similarly, epsin 1 (144-575) immobilized upon glutathione-Sepharose containing the DPW-binding AP-2 α appendage associates with higher-order polyubiquitin chains (Figure 2.10, lane d) while neither the immobilized α appendage nor GST interacts with the added ubiquitin (lanes b and f). These experiments demonstrate that epsin 1 physically bound either to clathrin or AP-2 can still associate productively with polyubiquitin but not monoubiquitin. Importantly, native epsin 1 and eps15, affinity isolated from brain cytosol using a GST-α appendage (R916A) mutant that selectively binds to these two endocytic proteins, associate with higher-order polyubiquitin chains but not with mono- or diubiquitin (Figure 2.11, lane f). This result is in accord with a recent study showing epsin 1 coimmunoprecipitates a group of uncharacterized ubiquitinated cellular proteins [152]. And lastly, when fused to GST, a ubiquitin-specific-protease-resistant linear tetraubiquitin efficiently affinity isolates endogenous epsin and eps15, as well as S5a and Hrs, from brain extracts (Figure 2.12, lane f). These proteins do not associate comparably with a single ubiquitin attached to GST (lane d), although the E1 ubiquitin-activating enzyme does bind GST-ubiquitin. Overall, we conclude minimally that epsin 1 is able to associate with polyubiquitin chains when simultaneously bound to other clathrin coat constituents such as AP-2 or clathrin.

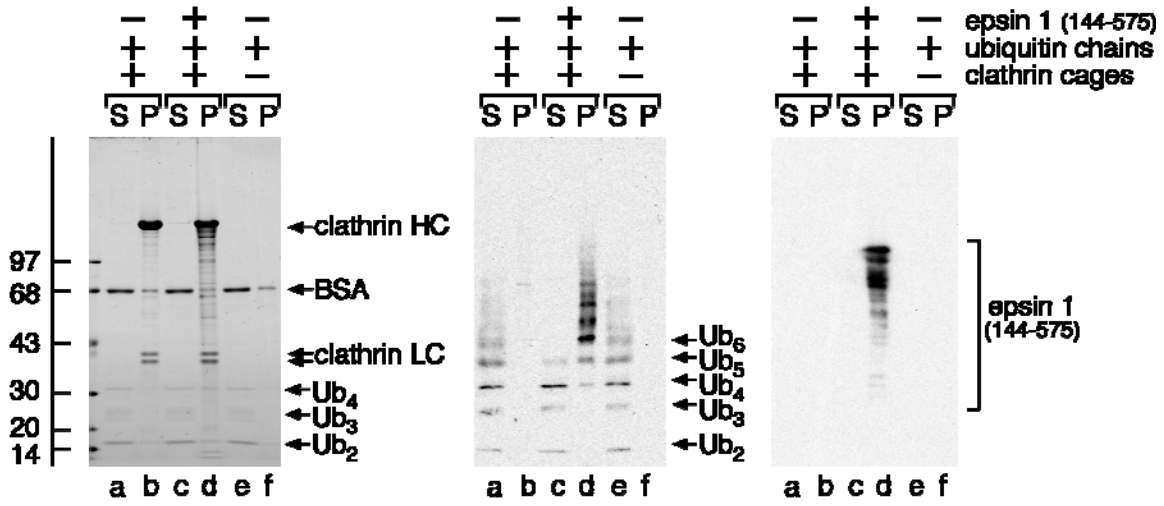

Figure 2.9: Incubation of epsin 1 with polyubiquitin chains and preassembled clathrin cages.
Mixtures of 25 μg preassembled clathrin cages and 0.1 mg/ml BSA with or without 15 mg His_6-epsin 1 (144-575) were preincubated and then recovered by centrifugation before a second incubation in the presence of 6 μg Lys48-linked polyubiquitin chains. After centrifugation, threefold more of each pellet (P) than each supernatant (S) was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin or anti-epsin antibodies. The recombinant epsin is prone to proteolytic degradation.

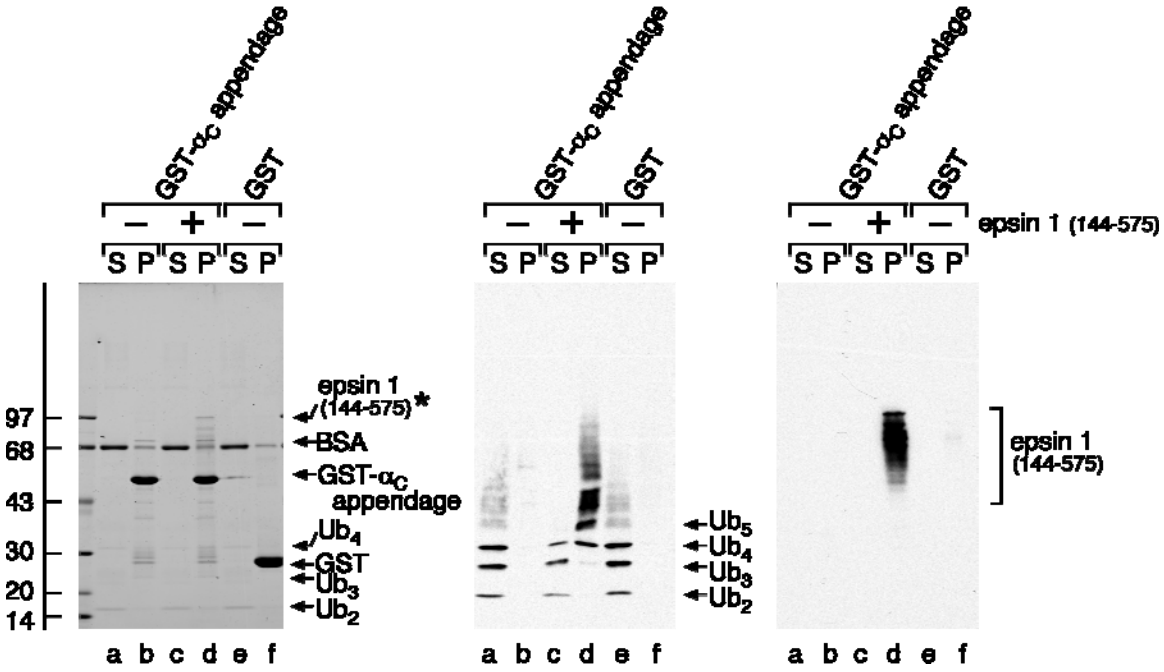

Figure 2.10: Binding of polyubiquitin chains to epsin 1 associated with AP-2 α appendage.
Binding of Lys48-linked polyubiquitin chains to 15 μg epsin 1 (144-575) associated with 25 μg immobilized GST or GST-AP-2 α_C appendage. After centrifugation, fivefold more of each pellet (P) than each supernatant (S) was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin or anti-epsin antibodies. The asterisk denotes the position of the intact epsin 1 (144-575) protein segment.

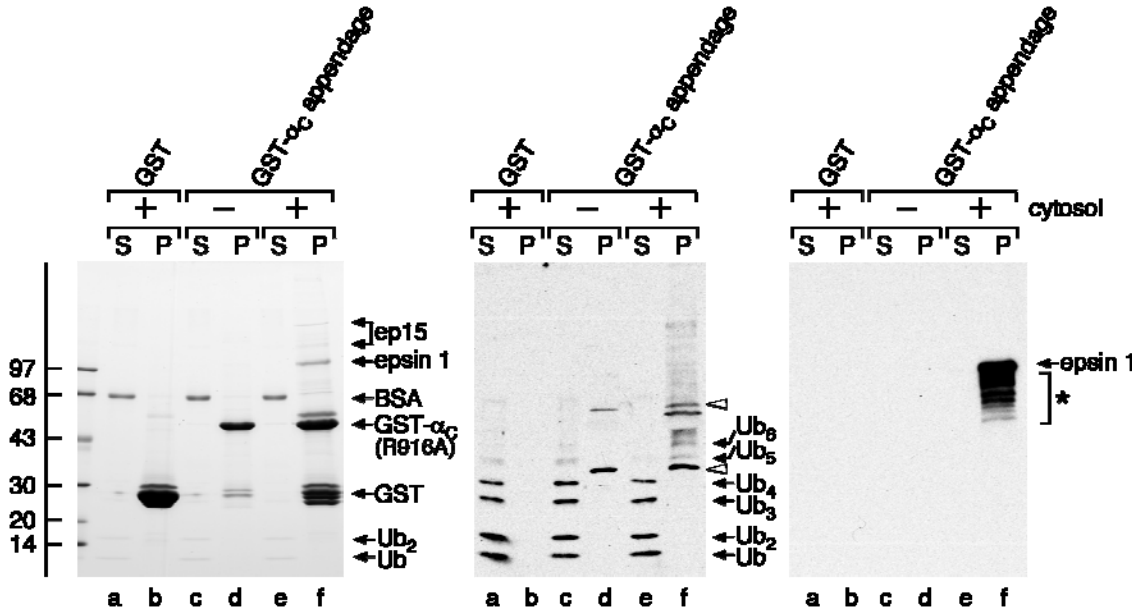

Figure 2.11: Binding of polyubiquitin chains to endogenous epsin 1 and eps15.
Binding of Lys48-linked polyubiquitin chains to endogenous epsin 1 and eps15 associated with 100 μg immobilized GST or GST-AP-2 α_C appendage (R916A). After centrifugation, ~7-fold more of each pellet (P) than each supernatant (S) was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with anti-ubiquitin, or anti-epsin antibodies. Open arrowheads indicate non-specific cross-reactive bands.

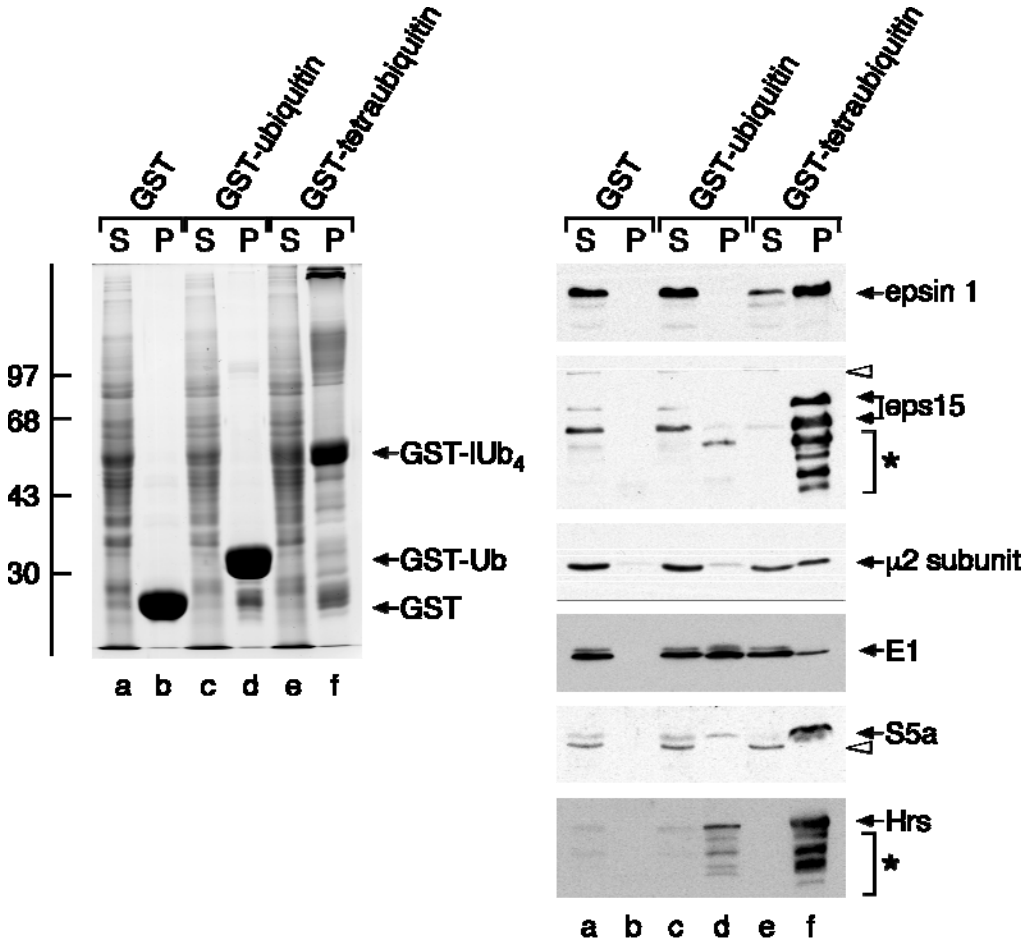

Figure 2.12: Binding of endogenous endocytic and ubiquitin components to various ubiquitin GST fusion proteins.

Binding of endogenous endocytic and ubiquitination components within brain cytosol to GST, GST-ubiquitin or a ubiquitin-protease-resistant linear GST-tetraubiquitin. After centrifugation, ~5-fold more of each pellet (P) than each supernatant (S) was resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with antibodies directed against the indicated proteins. The stained gel shows numerous proteins associate with the immobilized linear tetraubiquitin (lUb_4). Presumptive degradation products are indicated by the asterisks while non-specific cross-reactive bands, which remain in the supernatant (S), are indicated by the open arrowhead. The weak recovery of AP-2 most probably reflects a secondary association with the immobilized epsin 1 and eps15.

2.3.1 Subcellular Localization of Epsin and Eps15

Because epsin and eps15 have been suggested to sort ubiquitinated cargo into caveolae [145, 146], we examined the localization of these two proteins in several cell lines by both light and electron microscopy. The intracellular distribution of endogenous epsin 1 is highly coincident with AP-2 in NRK (Figure 2.13A-D), HeLa (Figure 2.14A-D), and A431 (Figure 2.15A-D) cells at steady state. In NRK cells, >87% of the epsin-positive structures contain AP-2, while >79% of AP-2 structures also stain for epsin 1. Colocalization occurs not only at the ventral plasma membrane, but also along the entire cell surface (Figure 2.13M). By contrast, two different antibodies against caveolin-1, a marker for caveolar/raft-dependent endocytic structures, fail to reveal substantial colocalization of caveolin-1 with either epsin 1 (<3%) or AP-2 (<8%) in NRK (Figure 2.13E-L) or HeLa (Figure 2.14E-H) cells. The spatial segregation of epsin from caveolae is confirmed by freeze-etch ultrastructural studies (Figure 2.13N). Despite high density labeling of polyhedral clathrin lattices on the surface with anti-epsin antibodies, caveolae, definitively identified by the characteristic whorl-like morphology, are essentially unlabelled. These data argue against epsin being a significant component of caveolae in these cells under normal conditions.

After serum starvation, stimulating cultured cells with high concentrations of EGF (> 5 ng/ml) is reported to promote epsin/eps15-dependent EGF receptor internalization within caveolae in addition to clathrin-coated vesicles [145]. Yet, after briefly stimulating A431 cells with saturating concentrations (100 ng/ml) [158] of EGF, there is little evidence of major redistribution of either epsin or eps15 from AP-2/clathrin-containing structures (Figure 2.15 and 2.16). A431 cells express millions of EGF receptors [159, 160] but we do not find major

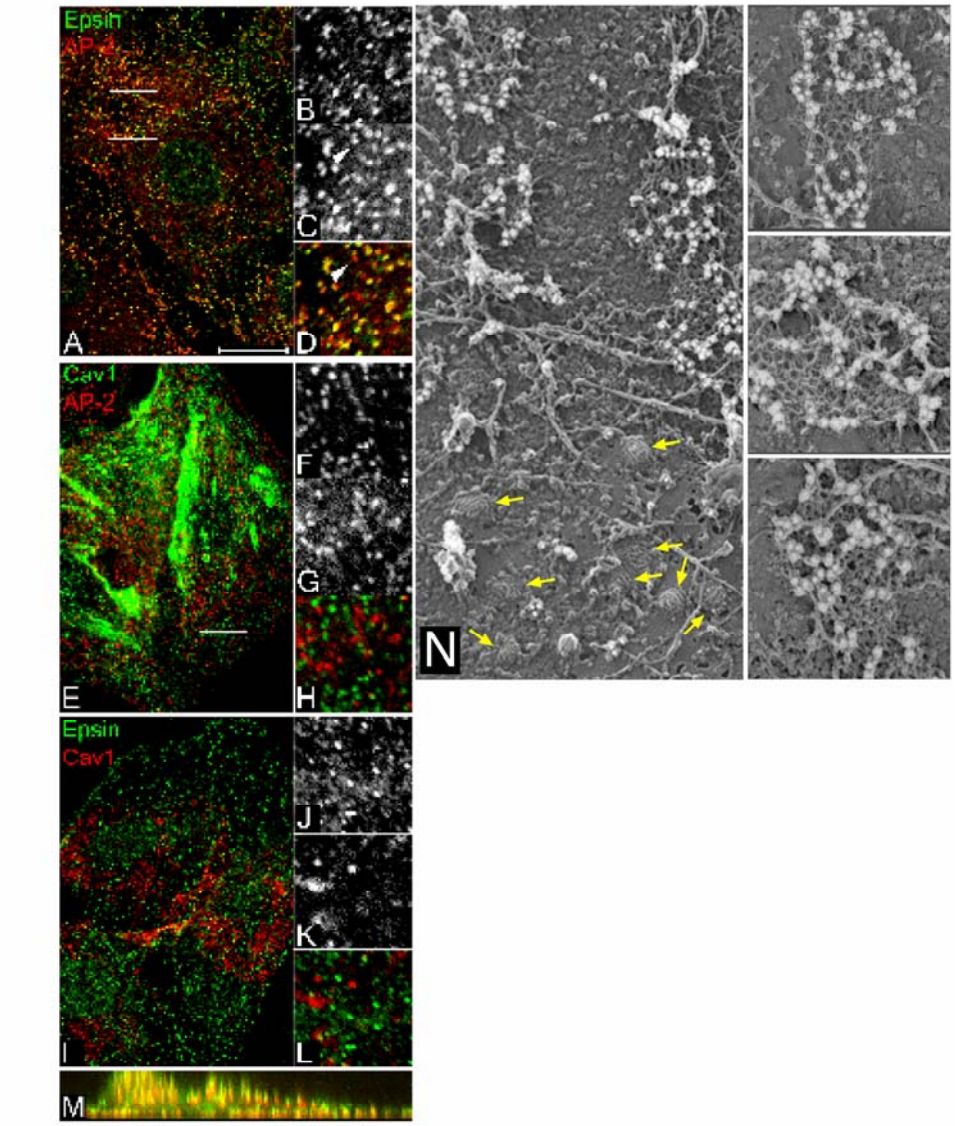

Figure 2.13: Epsin 1 is a component of the endocytic clathrin coat *in vivo*.
A-D) A representative single optical section of methanol-fixed NRK cells stained for epsin 1 (green) and AP-2 (α subunit, red). Magnifications of the boxed region in A are shown in B-D with the arrowhead indicating an AP-2-positive structure lacking epsin 1 staining. E-H) A representative single optical section of paraformaldehyde-fixed NRK cells stained for caveolin-1 (green) and AP-2 (α subunit, red). Magnifications of the boxed region in E are shown in F-H. I-L). A representative single optical section of methanol-fixed NRK cells stained for epsin 1 (green) and caveolin-1 (mAb, red). Magnifications of the boxed region in I are shown in J-L. M) X-Z projection of NRK cells stained with antibodies against epsin 1 (green) and AP-2 (α subunit, red). Scale bar is 10 μm. N) Freeze-etch analysis of NRK cell membranes stained with anti-epsin antibodies and 15 nm colloidal gold-conjugated secondary antibodies, visible as white spheres. Caveolae are indicated by the arrows on the left, and magnified views of three epsin/gold-containing clathrin lattices are shown in the right panels. Scale bar is 100 nm.

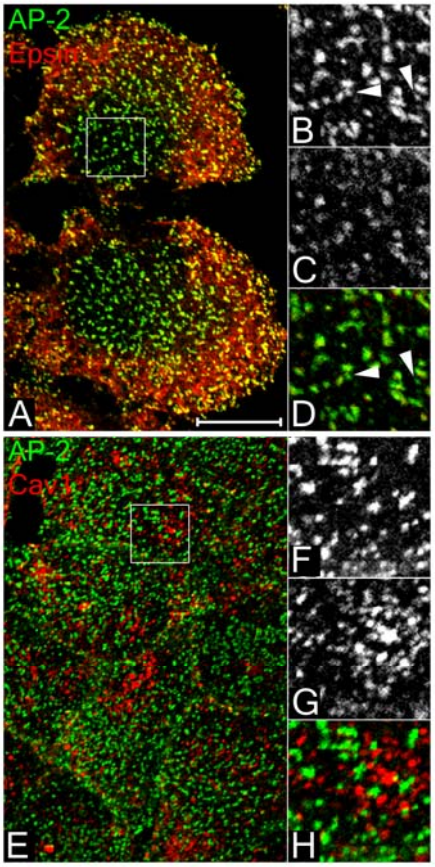

Figure 2.14: Absence of epsin 1 in caveolae in HeLa cells.
A representative single optical section of paraformaldehyde-fixed HeLa cells stained for AP-2 (α subunit, green) and epsin 1 (red). Magnifications of the boxed region in A are shown in B-D with the arrowheads indicating AP-2-positive structures lacking epsin 1 staining. E-H) A representative single optical section of paraformaldehyde-fixed HeLa cells stained for AP-2 (α subunit, green) and caveolin-1 (red). Magnification of the boxed region in E are shown in F-H**.** Scale bar is 10 μm.

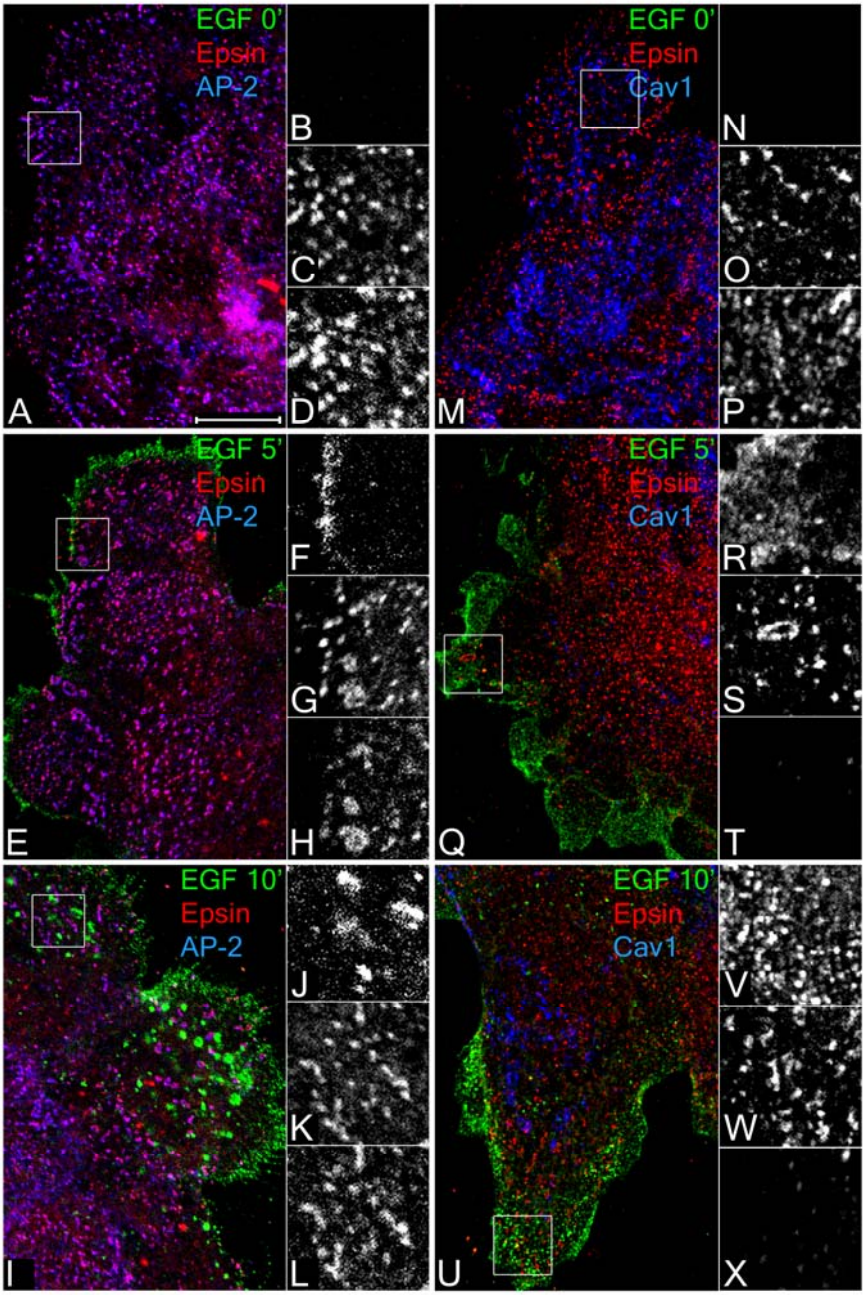

Figure 2.15: Absence of major reorganization of epsin upon EGF internalization in A431 cells.
Serum-starved A431 cells were incubated either without (A-D and M-P) or with 100 ng/ml Alexa488-conjugated EGF for 5 (E-H and Q-T) or 10 min (I-L and U-X) before fixation. A representative single optical section of the methanol-fixed A431 cells stained for epsin 1 (red) and either AP-2 (α subunit, blue) (A-L) or caveolin-1 (blue) (M-X) is shown. Magnifications of the boxed regions in A, E, I, M, Q, and U are shown in B-D, F-H, J-L, N-P, R-T, and V-X, respectively. Scale bar is 10 μm.

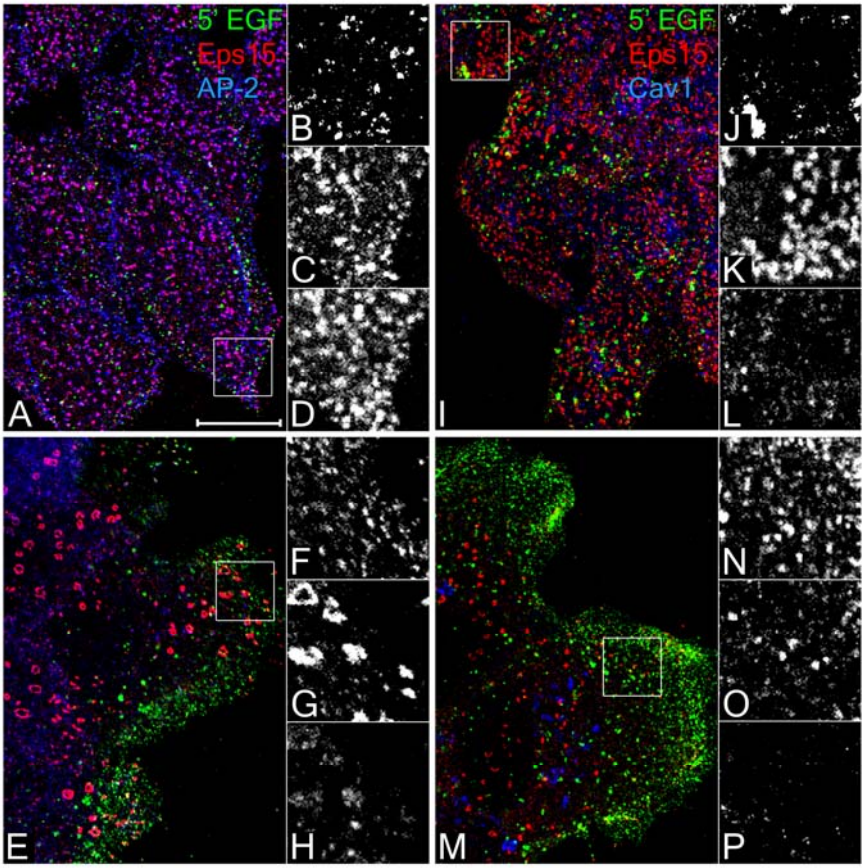

Figure 2.16: Co-localization of eps15 with AP-2 in EGF-stimulated HeLa and A431 cells.
Serum-starved HeLa (A-D and I-L) or A431 (E-H and M-P) cells were stimulated with 100 ng/ml Alexa488-EGF for 5 min prior to fixation. A single representative optical section of methanol-fixed cells stained for eps15 (red) and either AP-2 (α subunit, blue) (A-H) or caveolin-1 (blue) (I-P) is shown, with the magnifications of the boxed regions shown in on the right of each panel. Scale bar is 10 μm.

reorganization of either epsin or eps15 under conditions that stimulate internalization (Figure 2.15I and J) and extensive ubiquitination of EGF receptors. Less than 5% of either epsin 1 or eps15 colocalize with the caveolin-1 signal as opposed to ~80% coincidence of epsin and >90% of eps15 with AP-2 in A431 cells, both before and after EGF addition. Similarly, epsin (data not shown) and eps15 (Figure 2.16) remain extensively colocalized with clathrin-coated structures in HeLa cells treated with 100 ng/ml EGF. Thus, we find no evidence for bulk changes in cellular epsin 1 or eps15 localization or prominent redistribution during uptake of the EGF receptor; we favor the notion that these endocytic proteins appear to function chiefly in clathrin-mediated endocytosis. Our results are also concordant with recent observations showing that decorin-driven internalization and subsequent degradation of unactivated EGF receptors is caveolin dependent, but that ligand-activated EGF receptors enter the cell via clathrin-mediated endocytosis even at relatively high EGF concentrations [161]. They are also consistent with the recent finding that caveolae-dependent uptake of tight junction components in MDCK cells is insensitive to the effects of dominant-negative eps15 [162].

2.3.2 CLASPs and Ubiquitinated Cargo in Clathrin-Coated Vesicles

The biochemical experiments outlined above suggest that for designated cargo to be efficiently sorted by tandemly-arrayed UIMs, polyubiquitination rather than monoubiquitination might be required. To begin to analyze this issue, we first compared clathrin-coated vesicle preparations purified from either brain or placental tissue. Both populations of vesicles are highly enriched in the core clathrin-coat components, clathrin trimers, and AP-1/AP-2 adaptor heterotetramers (Figure 2.17). The brain-coated vesicles also clearly demonstrate, by altered electrophoretic

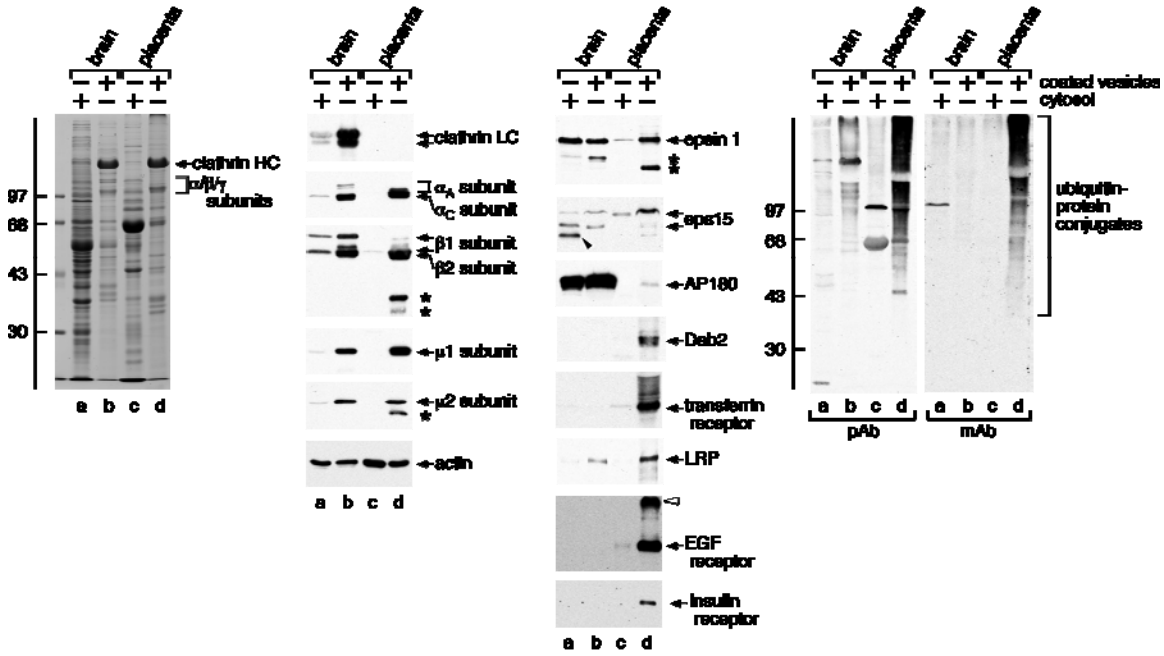

Figure 2.17: Cargo and clathrin-associated sorting protein enrichment in clathrin-coated vesicles from different tissues.

Samples of 25 μg of rat brain or placenta cytosol or 10 μg of purified clathrin coated vesicles were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose and probed with antibodies directed against the indicated proteins. Presumptive degradation products are indicated by the asterisks while, a non-specific cross-reactive band is indicated by the arrowhead. For the anti-EGF receptor immunoblot, the top of the gel is indicated by the open arrowhead. pAb is polyclonal antibody.

mobility, the occurrence of neural-specific isoforms of the basic clathrin coat machinery. Brain coated vesicles contain epsin, eps15, and AP180, although none of these proteins are enriched in parallel with core coat constituents. Strikingly though, the placental coated vesicles are significantly enriched in both cargo-selective CLASPs, and contain various cargo molecules including the transferrin, EGF, and insulin receptors and LDL receptor-related protein 1 (LRP1). The different representation of signaling and cargo receptors in these clathrin-coated vesicle preparations undoubtedly reflects the different overall function of these transport intermediates; at the synapse, coated vesicles are almost completely dedicated to retrieval of synaptic vesicle membrane and hence the paucity of signaling receptors [163].

Both epsin 1 and eps15 are enriched in the placental vesicles, and this correlates with the marked concentration of ubiquitin-protein conjugates in this preparation, as judged by both polyclonal anti-ubiquitin-protein conjugate and mAb anti-ubiquitin antibodies. Thus, there appears to be a cargo-selective enrichment of CLASPs in placental-coated vesicles, with the enrichment of UIM-bearing proteins correlating with the preferential inclusion of ubiquitin conjugates. It is intriguing that transcripts for two transmembrane RING-type E3 ubiquitin ligases, termed MARCH IV and IX, which are both capable of downregulating surface expression of major histocompatibility complex (MHC) class I molecules via ubiquitination, are very highly expressed in placenta [164].

2.3.3 Polyubiquitin Versus Monoubiquitin Triggered Endocytosis

To better understand whether polyubiquitin represents a more efficient internalization signal than monoubiquitin *in vivo*, the uptake of several chimeras of Tac (the IL-2 receptor α subunit or CD25) fused to ubiquitin (Figure 2.18A) was analyzed in transiently transfected HeLa cells.

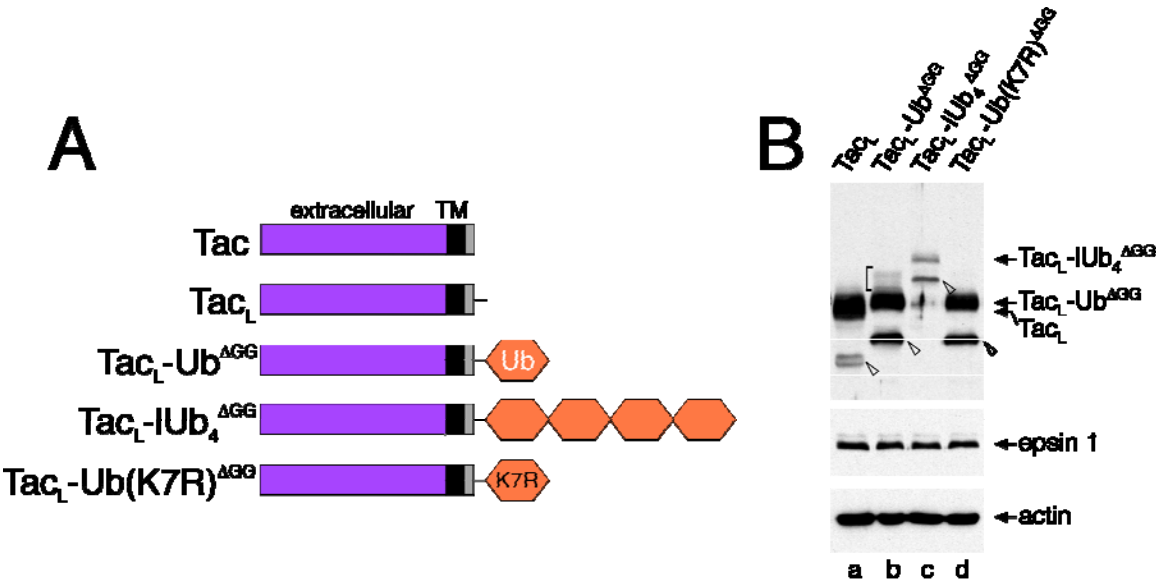

Figure 2.18: Schematic depiction of Tac and Tac-ubiquitin chimeras used and lysates of HeLa cells transfected with the chimeras.
A) Schematic depiction of the organization of Tac and the Tac-ubiquitin chimeras used. TM is transmembrane segment. B) Detergent lysates (~30 μg/lane) from HeLa cells transiently transfected with the indicated Tac constructs were resolved by SDS-PAGE, transferred to nitrocellulose and probed with antibodies directed against Tac, epsin 1 or actin. The immature glycosylated ER forms of Tac are indicated by the open arrowheads while the presumptive polyubiquitinated forms of Tac_L-$Ub^{\Delta GG}$ are bracketed.

Lysates from the transfected cells confirm expression of the various Tac proteins, oligosaccharide processing, and the stability of the linearly-linked tetraubiquitin adduct, which is expected to be generally oriented similarly to the extended Lys63-linked ubiquitin chain [165]. The overall abundance of the Tac_L-$lUb_4^{\Delta GG}$ is much lower than either the Tac_L or Tac_L-$Ub^{\Delta GG}$ (Figure 2.18B) and, similarly, surface expression of the Tac_L-$lUb_4^{\Delta GG}$ is generally substantially less than either the Tac_L or Tac_L-$Ub^{\Delta GG}$ proteins. Crucially, using surface-bound anti-Tac antibodies to follow internalization, Tac_L-$lUb_4^{\Delta GG}$ and Alexa488-transferrin are efficiently taken up into common endocytic structures upon warming to 37°C (Figure 2.19I-K). This indicates that the two transmembrane proteins, Tac_L-$lUb_4^{\Delta GG}$ and the transferrin receptor, follow a common clathrin-dependent trajectory into the cell interior. Frequently in the transiently transfected cells expressing Tac_L-$lUb_4^{\Delta GG}$, these two endocytic markers are located in exaggerated EEA1- and cation-independent mannose 6-phosphate receptor-positive endosomes, albeit within distinct endosome subdomains (Figure 2.19L,M). At 20 min, antibody uptake experiments show that a single ubiquitin-derivatized Tac (Tac_L-$Ub^{\Delta GG}$) is internalized poorly by comparison (Figure 2.19F-H), while Tac_L only appears to enter the cell at the basal rate of bulk membrane turnover (Figure 2.19C-E).

The TacL-$Ub^{\Delta GG}$ protein cannot be activated by the cellular E1 activating enzyme (because Gly75 or G76 required for conjugation are deleted), but additional ubiquitin molecules could still be conjugated to acceptor lysine residues present within the fused ubiquitin; indeed, several higher molecular-weight forms of the TacL-$Ub^{\Delta GG}$ are detected within lysates (Figure 2.18B). To evaluate whether conversion of the single ubiquitin to a polymeric chain affects internalization of the TacL-$Ub^{\Delta GG}$ protein, we also analyzed a Lys-for-Arg substituted TacL-$Ub^{\Delta GG}$ (TacL-Ub(K7R) $^{\Delta GG}$), which does not show the extra slower-migrating bands (Figure

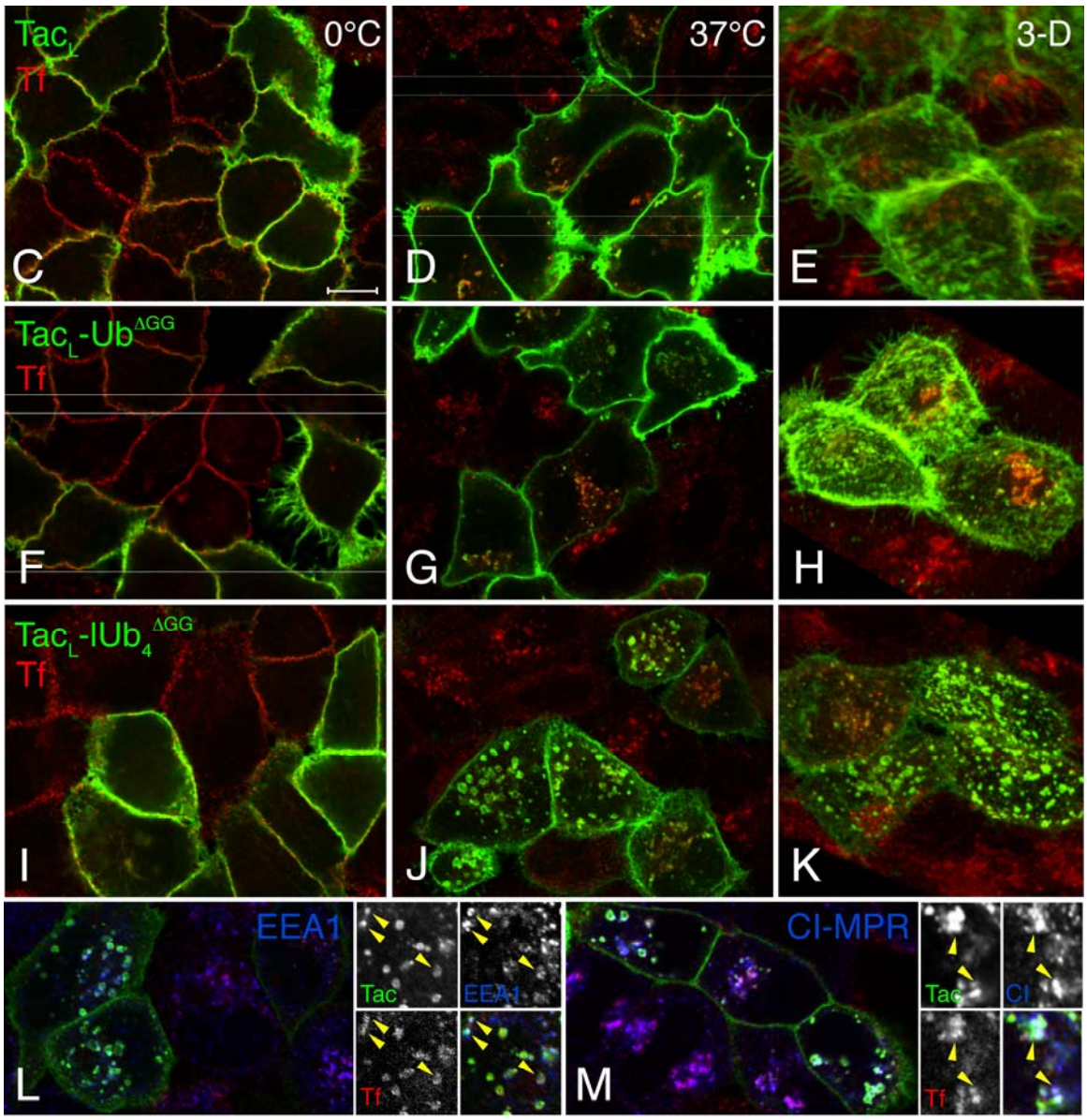

Figure 2.19: *In vivo* activity of polymeric ubiquitin as an endocytosis signal.
C-K) HeLa cells transiently transfected with Tac_L (C-E), Tac_L-$Ub^{\Delta GG}$ (F-H) or Tac_L-$lUb_4{}^{\Delta GG}$ (I-K) were incubated on ice with a mixture of 4 μg/ml anti-Tac mAb (green) and 25 μg/ml Alexa568-transferrin (red) for 60 min before warming to 37°C for 20 min. Representative single optical sections (panels C,D,F,G,I,J) or three-dimensional (3-D) reconstructions of X-Z image stacks (panels E, H, K) from fixed cells are shown. L-M) HeLa cells transiently expressing Tac_L-$lUb_4{}^{\Delta GG}$ as in I-J were stained for Tac (green), Alexa568-transferrin (red) or EEA1 (L, blue) or the cation-independent mannose 6-phosphate receptor (CI-MPR; M, blue). Color-separated magnified images of the mosaic Tac-positive endosomes (panel M) are shown. Scale bar is 10 μm.

2.18B). In short antibody uptake experiments, neither form of single ubiquitin fusion is rapidly internalized compared with the tetraubiquitin TacL-lUb4$^{\Delta GG}$ (Figure 2.20A-H). We suspect that the quantitatively minor polyubiquitinated forms of TacL-Ub$^{\Delta GG}$ seen on immunoblots (Figure 2.18B) are responsible for low-level Tac-Ub$^{\Delta GG}$ internalization observed (Figure 2.19 panel C and D). As numerous Tac-positive puncta accumulate in transfected cells after a 6-hour treatment with the lysosomal protease inhibitors leupeptin and pepstatin A (data not shown), we conclude that the reduced level of TacL-lUb4$^{\Delta GG}$ at the surface and within the cell reflects, in part, the rapid internalization of this construct and efficient, ubiquitin-dependent trafficking toward the lysosomal compartment. The appearance of aberrant endosomes in these cells is presumably related to the inability of the linearly-linked ubiquitin to be efficiently deubiquitinated by cellular isopeptidases. We believe that these experiments show clearly that a linear polyubiquitin chain is superior to an unextendable monoubiquitin in promoting endocytosis in cultured cells.

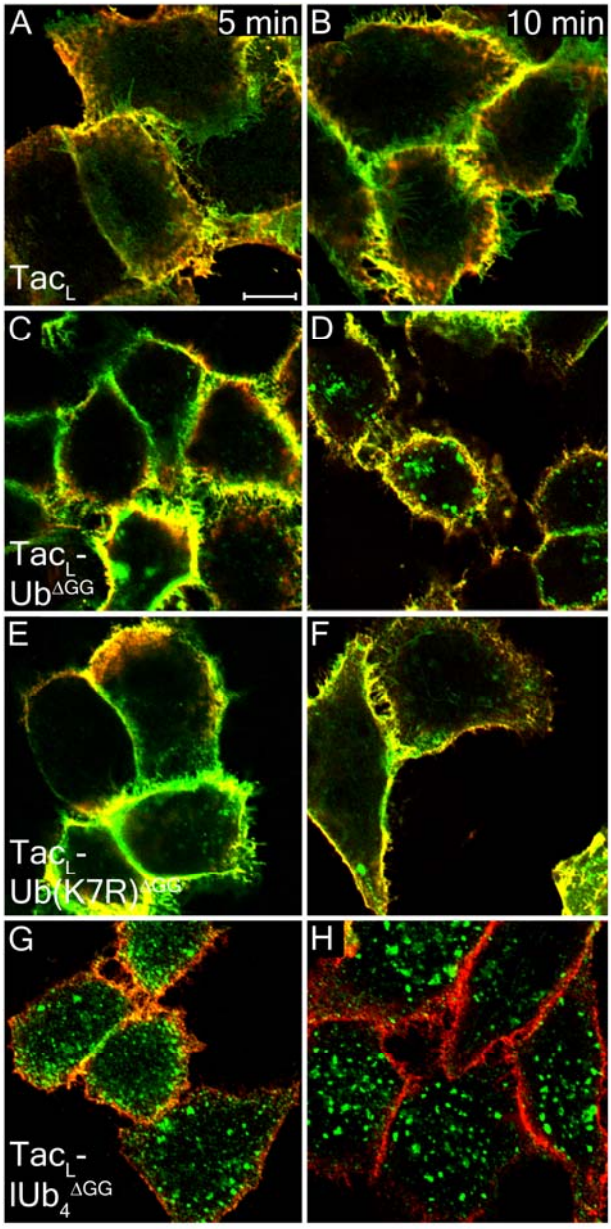

Figure 2.20: Comparison of mono- vs. polyubiquitin-conjugated Tac uptake.
HeLa cells transiently transfected with Tac_L (A, B), Tac_L-$Ub^{\Delta GG}$ (C,D), Tac_L-$Ub(K7R)^{\Delta GG}$ (E,F), or Tac_L-$lUb_4{}^{\Delta GG}$ (G,H) were incubated with 4 μg/ml anti-Tac mAb at 37°C for either 5 (A,C,E,G) or 10 min (B,D,F,H). After rapid cooling on ice, the cells were fixed and extracellular anti-Tac (red) detected with Alexa568-coupled anti-mouse IgG while intracellular anti-Tac (green) was labeled subsequently with Alexa488-conjugated anti-mouse IgG following saponin permeabilization. Representative single optical sections are shown and, because of the very high level of expression of the Tac_L, Tac_L-$Ub^{\Delta GG}$ and Tac_L-$Ub(K7R)^{\Delta GG}$ proteins and poor to negligible internalization, the Alexa488-conjugated secondary antibody also detects this surface pool (yellow). Scale bar is 10 μm.

2.4 DISCUSSION

In this study, we have plainly demonstrated that the preferred binding partner for the multiply-arrayed epsin 1 and eps15 UIMs is polyubiquitin, as is true for the UIMs of S5a [150]. In full accord with the grossly unstructured polypeptide main chain of the carboxy-terminal segment of epsin 1 [72, 157], we find that polyubiquitin engagement is still manifest when epsin is attached to a PtdIns(4,5)P_2-containing bilayer surface, AP-2, and/or clathrin, and that, under normal circumstances, epsin 1 and eps15 appear to be essentially absent from caveolar structures. Taken altogether, these results question the recent assertion that epsin and eps15 promote the internalization of monoubiquitinated cargo in a clathrin-independent fashion [145, 146]. Instead, in conjunction with the accompanying findings of Barriere et al. [166], we believe that these endocytic proteins are appropriately positioned within the assembling clathrin lattice to act as ubiquitin-selective CLASPs.

2.4.1 UIM Ubiquitin Selectivity

A single UIM is an amphipathic α-helical element (Figure 2.1B) that engages a hydrophobic surface upon the ubiquitin molecule generated in part by residues Leu8, Leu43, Ile44, Gly47, His68, and Val70 [155, 167-169]. Although different individual UIMs can associate with ubiquitin monomers with moderate affinity (K_d ~200-1000 μM), given the purported role of monoubiquitin as an internalization signal [125], the affinity for the UIMs is “surprisingly weak” [169]. Isolated UIMs are able to associate with ubiquitin-like (UbL) domains of proteins like HR23A with better affinity (K_d ~3-12 μM) [154, 155], but a valine substituted for ubiquitin residue His68 appears to be an important determinant of the higher affinity that UbL proteins

display for UIMs [154, 155]; and UbL domain lysines can be modified by E3 enzymes to create polyubiquitin chains anchored to the UbL [170, 171]. Our analysis shows that the fundamental binding properties of the epsin 1 (and eps15) UIM_3 segment are remarkably similar to those of a genuine polyubiquitin chain receptor S5a [150, 172], the protein that was used to identify the functional UIM domain [117]. Both bind robustly to polyubiquitin chains coupled by either Lys48 or Lys63 linkage. Lys63-linked chains are known to promote endocytosis in yeast [173] and facilitate proteasome-mediated proteolysis when appended to a model substrate [174]. Ubiquitin binding is cooperative [150]; tandemly arrayed epsin UIMs are required for efficient binding to polyubiquitin. This is consistent with endocytosis in *S. cerevisiae*, where Vps27p and Hse1p are mutually interacting, UIM-harboring sorting molecules that aid in the ubiquitin-dependent involution of designated cargo into the lumen of multivesicular bodies [175]. Vps27p contains two UIMs and both are needed for ubiquitin binding, while Hse1p contains only a single UIM and, alone, is unable to bind ubiquitin [175]. Other multiple UIM-containing proteins are also judged to preferentially bind polyubiquitin chains [176, 177]. Most importantly, though, we find that a single unextendable ubiquitin is insufficient to promote rapid endocytosis. A linear, ubiquitin-protease-resistant chain is far more effective at driving uptake from the cell surface.

Ongoing molecular dissection of the ubiquitin–proteasome system in *S. cerevisiae* indicates that there may be two (or more) parallel tracks for substrate delivery to the proteasome [172, 178]. The dichotomy reflects, in part, the size of the appended polymeric ubiquitin tag. Longer chains utilize Rpn10p (S5a) to engage the 19S complex while shorter chains appear to use the UbL/UBA proteins Rad23p and Dsk2p for targeting to the proteasome [178]. Yet, Rpn10p and Rad23p can operate redundantly in the degradation of certain model substrates [172]. Irrespective, the minimum chain length necessary to specify efficient proteasomal

degradation is four to six covalently-conjugated ubiquitins [99, 150, 178, 179]. This is in general accord with our observations and suggests that it seems unlikely that an as yet uncharacterized endocytic UbL/UBA protein, such as hPLIC/ubiquilin, governs the recognition of monoubiquitinated cargo at the clathrin bud site [171].

Because available structural information shows that the UIM helix interfaces with only a single ubiquitin monomer [151, 155, 167-169], and as Lys48- and Lys63-linked ubiquitin polymers have different geometries [165, 180], we do not invoke the recognition of a unique structural feature of polyubiquitin chains by the UIM, as is seen with the Lys48-linked polyubiquitin and HR23A [180] or Mud1p [181] UBA domains. Instead, our interpretation of the current information is that avidity effects allow polyubiquitinated cargo molecules to engage UIM-containing CLASPs massed at clathrin assembly zones on the surface [151]. These observations also rationalize the evolutionary conservation of the polyubiquitin-binding UIM amongst regulatory components of the proteasome and clathrin-coat machinery [117]. On the basis of reactivity with mAb FK1, which apparently detects polyubiquitin chains but neither monoubiquitinated proteins nor free ubiquitin [182], or experiments with K7R-type ubiquitin, several receptors (particularly receptor tyrosine kinases) appear to be multiply monoubiquitinated [122, 183, 184]. Avidity-driven recognition of ubiquitin by UIMs (in *cis* or *trans*) positioned within the clathrin assembly zone is also compatible with selection of multiply monoubiquitinated receptors. While an individual monoubiquitination event or the in-frame fusion of a single ubiquitin may be sufficient for endocytosis of certain receptors, even in *S. cerevisiae*, where ubiquitin represents the major endocytic signal [38, 125, 147], multiubiquitin does seem to be appended to Ste2p under normal conditions [122] and, like other heptahelical G protein-coupled receptors, Ste2p might also dimerize upon ligand binding.

Our current work does not address monoubiquitination of epsin and eps15 and how this contributes to the overall operation or regulation of clathrin-mediated endocytosis, but it should be considered that the general failure to detect heavily polyubiquitinated cargo molecules in biochemical experiments could be related, in part, to the catalytic activity of abundant cellular deubiquitinating enzymes. In this regard, there exist modular josephin domain-containing deubiquitinating enzymes, such as ataxin-3, that also contain tandem UIMs to target them to polyubiquitin chains [176, 177]. Still other deubiquitinating isopeptidases, such as mUBPY that associates indirectly with the EGF receptor [185], could also modulate the ubiquitination state of receptors.

2.4.2 A Poly/Multiubiquitin-Based Endocytic Sorting Signal

In considering a role for ubiquitin chains as an endocytic signal, it is worth noting that Rsp5p, the *S. cerevisiae* HECT-type E3 ubiquitin ligase required for endocytosis [186] that is structurally and functionally related to vertebrate Nedd4, is clearly able to preferentially synthesize Lys63-linked polyubiquitin chains [187], and conjugation-competent Lys63 ubiquitin is required for efficient endocytosis of some transmembrane proteins in yeast [173, 188]. It has also just been reported that Lys63-linked polyubiquitination drives efficient internalization of the TrkA receptor in mammalian cells [189]. Significantly, an Rsp5p-associated deubiquitinating enzyme termed Ubp2p disassembles Lys63-linked polyubiquitin chains [187]. Thus, complex cycles of ubiquitination/deubiquitination appear to be subject to tight temporal regulation intracellularly. Suitably positioned deubiquitinating isopeptidases could cleave polymeric ubiquitin from cargo molecules promptly following cargo selection, making identification and isolation of extensively ubiquitinated proteins difficult. Likewise, the extensive

*poly*ubiquitination that triggers endocytosis of the TrkA and TrkB receptor tyrosine kinases is negatively modulated by the p75 neurotrophin receptor [190].

Strong functional evidence for polyubiquitin acting as a physiological and direct internalization tag comes from the action of viral-encoded, RING variant-type transmembrane E3 ubiquitin ligases that very efficiently downregulate surface expression of host cell transmembrane immunoreceptors [191-194]. These E3s robustly polyubiquitinate MHC class I, B7.2, ICAM-1, and CD1d to rapidly remove these transmembrane proteins from the surface of virus-infected cells, and a polypeptide ladder revealing the posttranslational addition of at least four ubiquitin monomers is clearly visible on MHC class I molecules [193]. Given the physiological role of these virally encoded E3 ligases, they are unlikely to have evolved binding sites for cognate deubiquitinating enzymes and this, along with the concentration and catalytic activity of the ligases, may explain why polyubiquitinated transmembrane substrates are easier to detect in cells expressing these E3s or infected with virus.

Further compelling genetic and functional evidence of the importance of ubiquitination in promoting endocytic uptake comes from the Notch signaling pathway in *Drosophila*. The S3 proteolytic cleavage event necessary for the translocation of the signaling Notch intracellular domain into the nucleus depends upon the co-internalization of the extracellular region of Notch, bound to the transmembrane ligand Delta, into the Delta-expressing signal-sending cell [195]. Endocytosis of Delta is both ubiquitin and epsin dependent [196-198]. Two RING-type E3 ubiquitin ligases, Neuralized and Mind bomb, can ubiquitinate Delta as a prelude to clathrin-mediated endocytosis governed by Liquid facets [196-198], the *Drosophila* epsin orthologue [199]. Substitution of the endogenous Delta cytosolic domain with a portion of the cytosolic region of the LDL receptor containing an FXNPXY internalization motif negates the requirement

for Liquid facets in Delta operation [200]. This attests to the active role that ubiquitination plays in receptor uptake and argues that regulated ubiquitination clearly represents a reversible internalization signal. Results from the *Drosophila* system are also consistent with the poor capability of a single ubiquitin chain to promote efficient endocytosis. Within wing imaginal discs, clones expressing a form of Delta with the cytosolic domain replaced with a single ubiquitin molecule are only able to drive the expression of the early-acting Notch target Vestigial boundary enhancer, and not downstream Notch-activated proteins like the homeodomain protein Cut, or the secreted morphogen Wingless at the developing wing margin [200]. This is in contrast to the Delta-LDL receptor chimera, or a Delta receptor with a random, lysine-containing, polypeptide region substituted for the natural cytosolic domain; clones expressing either of these Delta constructs are able to activate Cut expression [200]. Taken together with our results, and those in the accompanying studies by Barriere et al. [166], we believe there is now strong functional evidence that epsin, together with eps15, operates as a CLASP capable of recognizing and packaging poly/multiubiquitin-based internalization signals into clathrin-coated vesicles forming at the cell surface of mammalian cells.

2.5 ADDITIONAL DATA

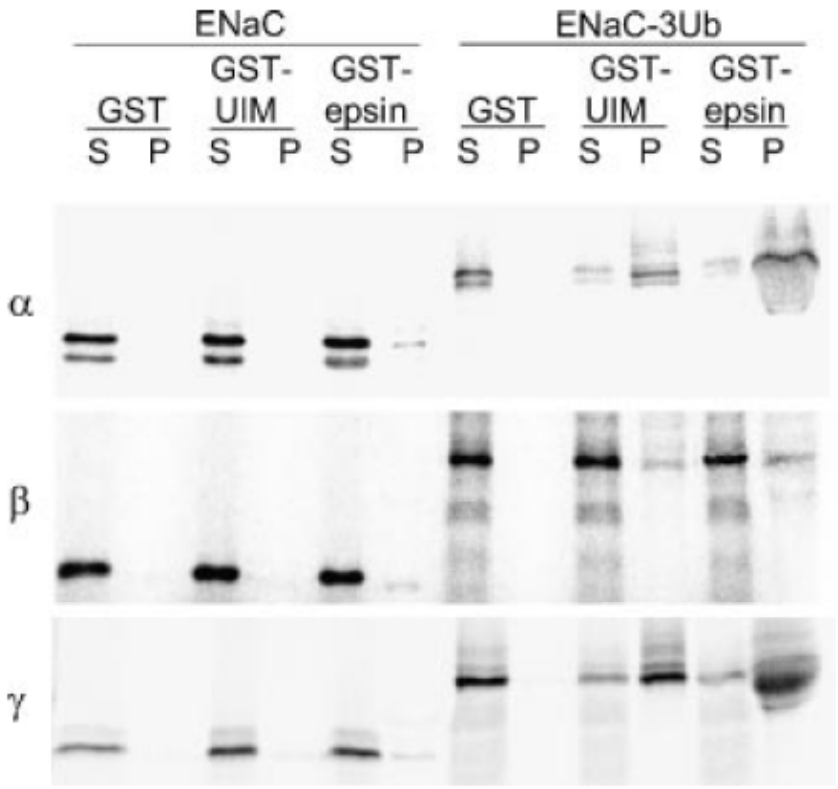

Figure 2.21: ENaC interaction with epsin UIM domains is enhanced by their conjugation to ubiquitin. Each ENaC subunit alone, or conjugated to three tandem ubiquitins was *in vitro* translated in the presence of [^{35}S] methionine, and equal amounts of isotope were then incubated with GST, GST-UIM, or GST-epsin constructs as described under "Materials and Methods." Samples were then incubated with glutathione-Sepharose beads and the beads were spun down. Samples from both pellet (P) (bound to beads) and supernatant (S) were then separated by SDS-PAGE and analyzed on a phosphorimager. Ubiquitin conjugated β–ENaC conjugate aggregated on entry to the 10% SDS gels and did not fully migrate, therefore, ENaC samples were separated on 8% SDS-PAGE with 8M urea. *In vitro* translated α, β, and γ ENaC were visualized at 79, 72, and 74 kDa, respectively, and were largely recovered in supernatant. There is a slight background interaction between unmodified ENaC and both GST and GST-UIM, and all three subunits have a weak interaction with GST-epsin, but the majority of the *in vitro* translate is in the supernatant. ENaC fused to ubiquitin runs typically higher in the gels as seen with α, β, and γ subunits visualized at 107, 100, and 102 kDa respectively. All three ENaC subunits fused to 3 tandem ubiquitins show significant accumulation in the pellet of GST-UIM and GST-epsin, indicating an interaction (n=3).

3.0 DUAL-ENGAGEMENT REGULATION OF PROTEIN INTERACTIONS WITH THE AP-2 ADAPTOR α APPENDAGE*

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3.1 ABSTRACT

Clathrin-mediated endocytosis depends upon the coordinated assembly of a large number of discrete clathrin-coat components in order to couple cargo selection with rapid internalization from the cell surface. Accordingly, the heterotetrameric AP-2 adaptor complex binds not only to clathrin and select cargo molecules, but also to an extensive family of endocytic accessory factors and alternate sorting adaptors. Physical associations between accessory proteins and AP-2 occur primarily through DP[FW] or FXDXF motifs, which engage an interaction surface positioned on the carboxy-terminal platform subdomain of the independently folded α-subunit appendage. Here, we define structurally and functionally a second interaction site on the bilobal α appendage, located on the amino-terminal β-sandwich subdomain, that binds to the WXX[FW]X[DE] interaction motif found in several endocytic proteins, including synaptojanin 1, stonin 2, AAK1, GAK and NECAP 1. Both α appendage binding sites can be engaged

synchronously, and our data reveal that varied assemblies of interaction motifs with different affinities for two sites upon the α appendage can provide a mechanism for temporal ordering of endocytic accessory proteins during clathrin-mediated endocytosis.

3.2 INTRODUCTION

Clathrin-coated vesicles are a major portal of entry into eukaryotic cells, carrying macromolecular nutrients, ligands, select transmembrane proteins, and even viruses into the cell interior from the plasma membrane [1, 68]. Cargo selectivity of these short-lived transport intermediates is often thought to be governed by a central triad of proteins, the cargo receptors, clathrin, and the heterotetrameric AP-2 adaptor complex. Cargo receptors contain cytosolic internalization sequences, such as the YXXØ motif (where X is any amino acid and Ø represents a residue with a bulky hydrophobic side chain) found within proteins like the receptor for the endocytosed iron transport protein transferrin. Several distinct internalization sequences or tags are known, each specifying internalization by promoting preferential incorporation into assembling clathrin-coated vesicles [38]. Clathrin functions as a trimer of heterodimers, composed of three 192-kDa heavy and three 20-25-kDa light chains, that polymerizes to form the characteristic polyhedral clathrin lattice [45, 201]. Assembled clathrin appears to act as a mechanical scaffold during the process of bud invagination. AP-2, the archetypical sorting adaptor, is composed of two large (~100 kDa) subunits (α and β2), a 50-kDa medium μ2 subunit and a 17-kDa small σ2 chain [55, 68]. AP-2 binds physically to both clathrin, through the hinge and appendage domains of the β2 subunit [70], and to YXXØ-type internalization sequences, via

the μ2 subunit, in a phosphorylation-regulated manner [54, 55, 58, 59]. AP-2 is therefore a multifunctional protein that couples coat assembly with cargo selection.

Surprisingly, after siRNA silencing of either the AP-2 α or μ2 subunit mRNA in HeLa cells to deplete cellular AP-2 adaptor levels, certain transmembrane receptors, like the EGF and LDL receptors, still internalize efficiently in a clathrin-dependent manner [95]. This demonstrates that AP-2 is not absolutely essential for clathrin-mediated endocytosis in cultured mammalian cells. Yet, siRNA knockdown of AP-2 decreases the abundance of clathrin-coated structures at the cell surface >90% [95], and severe mutation AP-2 α subunit in *Drosophila melanogaster* [94] or targeted disruption of the μ2 subunit genes in mice (J. Bonifacino, pers. comm.) is lethal. Thus, the AP-2 adaptor does play a pivotal role in clathrin coat dynamics at the plasma membrane.

In addition to binding YXXØ-type internalization sequences, AP-2 also binds, via the independently folded α and β2 subunit appendages that project off the heterotetrameric core, to at least twelve endocytic accessory proteins and alternate adaptors [48, 68, 201]. These interactions depend upon short interaction motifs or ligands often tandemly arrayed in structurally disordered segments of the AP-2 binding proteins. Two discrete sequences, the DP[FW] and FXDXF motifs, bind to a partially overlapping site on the α appendage [72]. Several endocytic proteins contain both of these, as well as a recently identified third α appendage binding sequence, the WXX[FW]X[DE] motif [82, 135, 202]. While these interaction motifs seem responsible, in part, for placement of endocytic accessory proteins and alternate adaptors at bud sites on the plasma membrane, how the complex web of protein-protein interactions is regulated, how temporally-ordered recruitment is achieved, and the physiological benefit of one type of interaction motif over another is currently unknown. In this study we show

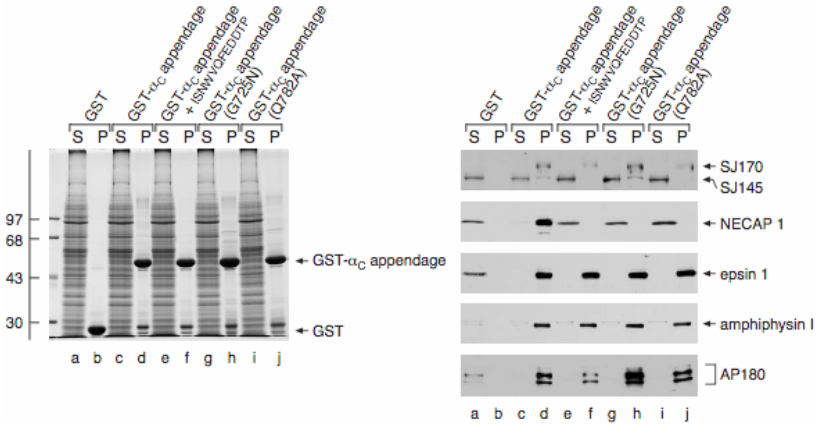
that the WXX[FW]X[DE] motif engages a Trp-specific binding site on the β-sandwich subdomain of the appendage that is distant from the major DP[FW]/FXDXF binding site on the platform subdomain of the α_C appendage. The sandwich site increases the number of α_C appendage binding modes and we propose a model for hierarchical protein recruitment based on the representation of different interaction motifs with different affinities positioned within intrinsically disordered domains of endocytic α_C appendage binding proteins.

3.3 RESULTS

3.3.1 A Second Functional α_c Appendage Binding Site

The WXX[FW]X[DE] motif occurs in several endocytic proteins, including synaptojanin 1, a phosphoinositide polyphosphatase, the Ser/Thr kinases AAK1 and GAK/auxilin 2, which control YXXØ-type internalization sequence binding through adaptor μ subunit phosphorylation, and in stonin 1/2 and NECAP 1/2, the precise functions of which are currently unknown [82, 135, 202]. Like the DP[FW] and FXDXF motifs, the WXX[FW]X[DE] motif binds physically to the AP-2 α appendage [82, 135, 202]. Yet, in affinity interaction assays *in vitro*, addition of a 250-fold molar excess of a stonin 2-derived ^{90}ISN**W**VQ**F**EDDTP peptide, which effectively prevents NECAP 1 binding and significantly blunts SJ170 interactions with GST-α_C appendage (Fig. 3.1A, lane f compared to lane d), has little effect on the association epsin 1 or amphiphysin I with the immobilized appendage [135, 202]. This finding suggests that the WXX[FW]X[DE] motif might be accommodated by a separate site upon the α appendage. Because the

A.



B.

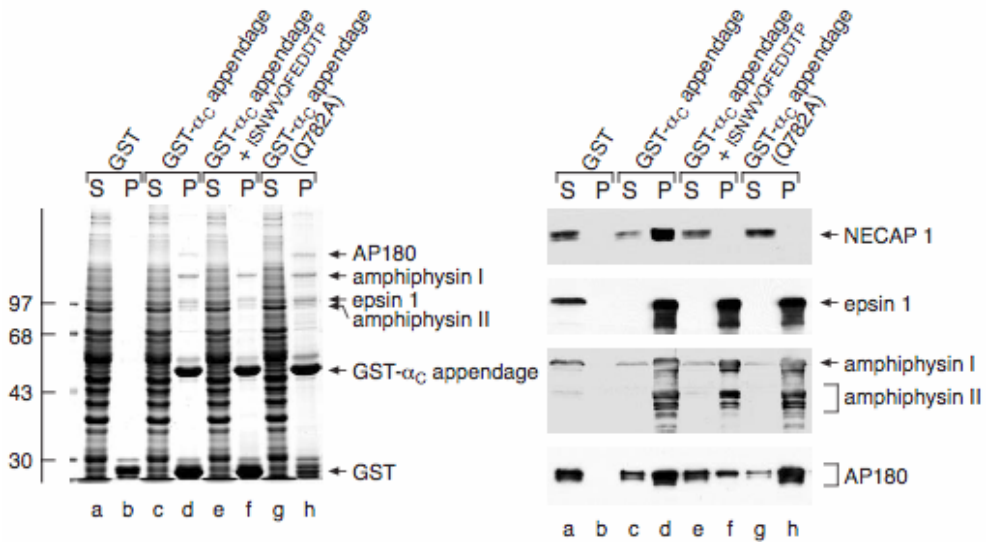


Figure 3.1: Separable protein interactions with the AP-2 α appendage.
(A) Approximately 50 μg of either GST (lane a and b), GST-α_C appendage (lane c -f), GST-α_C appendage (G725N) (lane g and h) or GST-α_C appendage (Q782A) (lane i and j) immobilized on GSH-Sepharose was incubated with PC12 cell lysates in the absence or presence of 3 mM ISNWVQFEDDTP peptide. After centrifugation, aliquots corresponding to 1/40 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed with the anti-synaptojanin 1 antibody AR/1, or anti-NECAP 1 or epsin 1 antibodies, or an anti-amphiphysin or -AP180 mAb. The position of the molecular mass standards (in kDa) is indicated on the left and only the relevant portion of each blot is shown. The different forms of AP180 in the PC12 cell lysates could be due to alternatively spliced isoforms or different posttranslational modifications. (B) Approximately 50 μg of either GST (lane a and b), GST-α_C appendage (lane c -f), or GST-α_C appendage (Q782A) (lane g and h) immobilized on GSH-Sepharose was incubated with rat brain cytosol in the absence or presence of 3 mM ISN**W**VQ**F**EDDTP peptide. After centrifugation, aliquots corresponding to 1/60 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed anti-NECAP 1 or epsin 1 antibodies, or an anti-amphiphysin or -AP180 mAb. The identity of the Coomassie-blue stained α_C-appendage binding partners is indicated and validated by the immunoblots on the right.

WXX[FW]X[DE] motif has an absolute requirement for Trp at the 0 position [82], we examined the contribution of residues located on the sandwich subdomain of the α_C appendage that we showed previously create a Trp-specific binding site (Fig. 3.2A-C) [72]. Purified, monomeric α_C appendage binds to a minimal SJ170-derived (^{1478}SNPKG**W**VT**F**EEE; GST-SJ170M1) WXX[FW]X[DE] motif immobilized on GSH-Sepharose beads (Fig. 3.3). The majority of the α_C appendage sediments with the GST-SJ170M1 fusion (lane d) while the appendage remains soluble (lane a) in the presence of GST alone (lane b). Alteration of select residues on one face of the amino-terminal sandwich, composed of β strands A', A, B, and E, perturbs the association of the α_C appendage with the immobilized WXX[FW]X[DE] motif. The effect of a Q782A substitution is most severe, while a G725N mutation also blunts appendage binding significantly (Fig. 3.4). Based on the structure of the apo-α_C appendage [69, 203] (Fig. 3.2B), and an epsin 1-derived DPW peptide co-crystallized at this site (Fig. 3.2A) [72], we suspect that Gln782 accepts a hydrogen bond from the indole nitrogen of the WXX[FW]X[DE] Trp 0, accounting for the strict Trp selectivity [82]. The G725N substitution likely fills much of the pocket required to accommodate the Trp residue (Fig. 3.2C). Altering several other side chains on the sandwich (Table 3.1) reveals that Phe740 is also required for productive interactions with the SJ170 WXX[FW]X[DE] model protein while a K727A substitution has negligible effect (Fig. 3.3). Several other side chains in the vicinity of the sandwich binding pocket do not affect appendage binding in this assay when mutated (Table 3.1).

The spatially distinct WXX[FW]X[DE] binding site on the sandwich subdomain is verified by ITC experiments. A synthetic KG**W**VT**F**EE peptide corresponding to the SJ170 WXX[FW]X[DE] motif interacts with the wild-type α_C appendage with a K_d of 10.7 ± 0.3 μM (Fig. 3.4 & 3.5). Mutations to the platform subdomain which have an affect on DPF (R905A, K_d

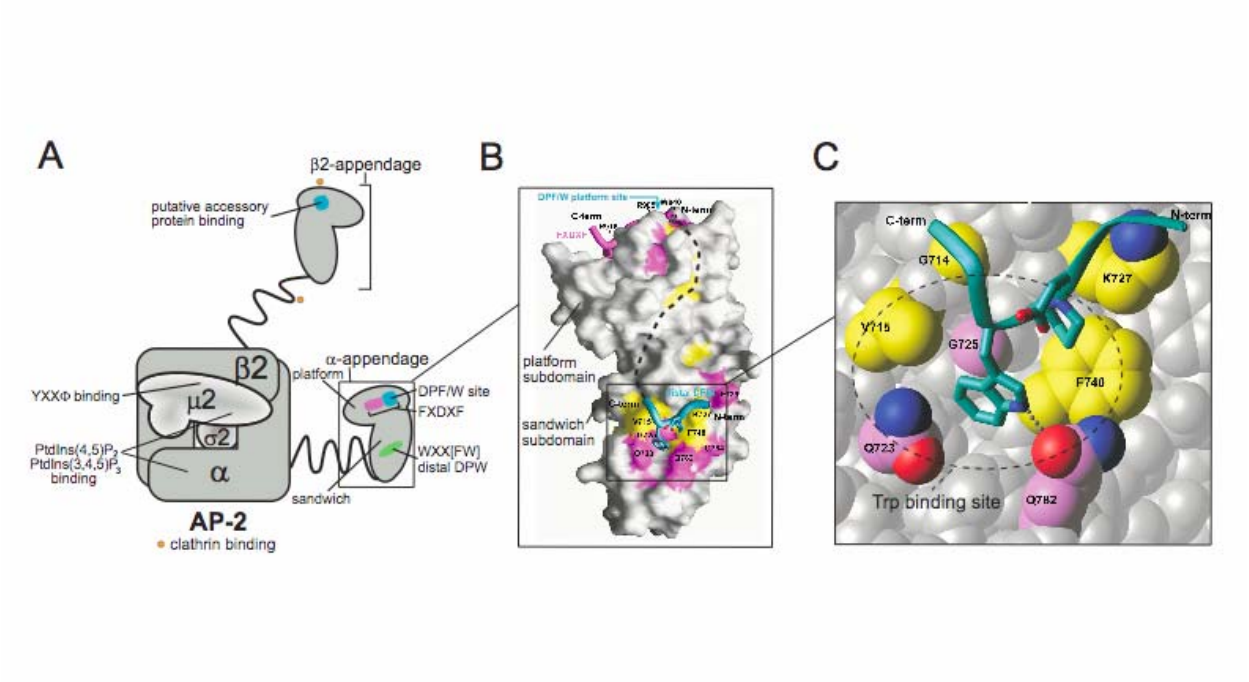


Figure 3.2: AP-2 schematic with a surface representation of the α appendage.
(A) Schematic of the AP-2 adaptor based on the crystal structure of the heterotetrameric AP-2 core and showing the various protein- and phospholipid-interacting regions. (B) Surface representation of the AP-2 α_C appendage showing the relative position of the platform binding site and the sandwich subdomain distal DPW surface containing a Trp-accommodating site. The surface is colored according to sequence conservation (magenta is invariant and yellow exhibit a conservation index of 7 or higher as scored by ALSCRIPT). Worm representations of the FXDXF and DPW peptides are shown in the platform and distal DPW sites as determined in our previous structural studies. The dashed line represents a hypothetical polypeptide chain connecting engagement of the two sites in concert. (C) Close-up view of the Trp-selective pocket on the sandwich subdomain. The position of several side chains examined in this study is indicated with C atom conservation colored as in Figure 3.2B. The position of the DPW peptide is displayed in worm form to indicate the Trp binding site.

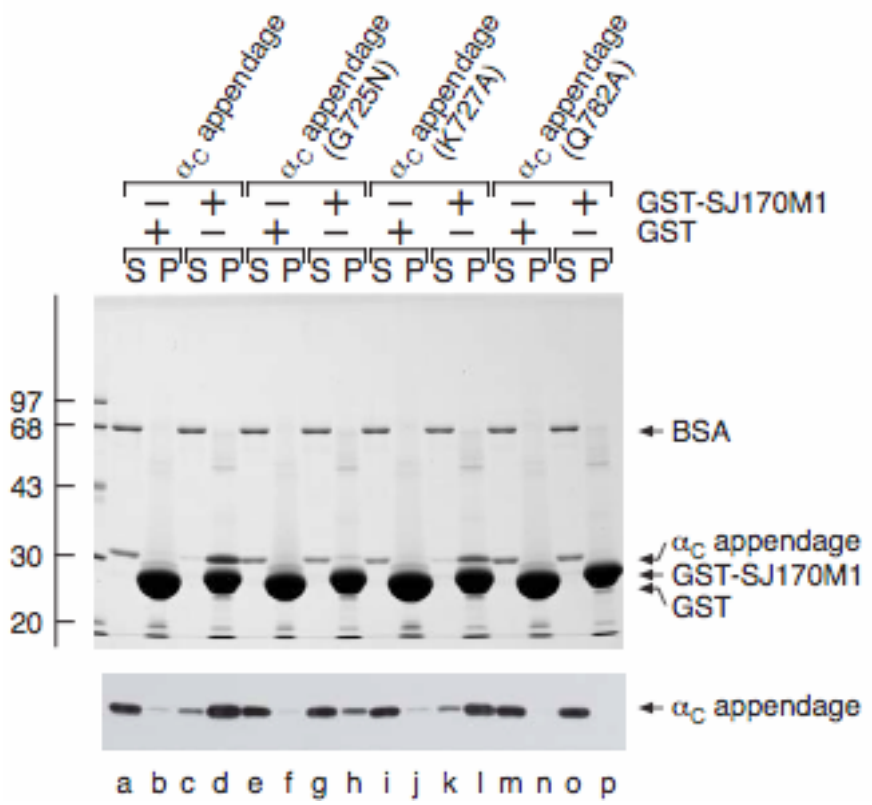


Figure 3.3: A tryptophan-specific WXXF-binding site on the α appendage.
Approximately 400 μg of either GST (lane a, b, e, f, i, j, and m and n) or GST- SJ170M1(lane c, d, i and j) immobilized on GSH-Sepharose was incubated with either thrombin-cleaved, monomeric α_C appendage (lane a-d), or α_C appendage (G725N) (lane e-h), or α_C appendage (K727A) (lane i-l), or α_C appendage (Q782A) (lane m-p) in the presence of carrier BSA. After centrifugation, aliquots corresponding to 1/40 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. The blot was probed with the anti-AP-2 α-subunit mAb 100/2.

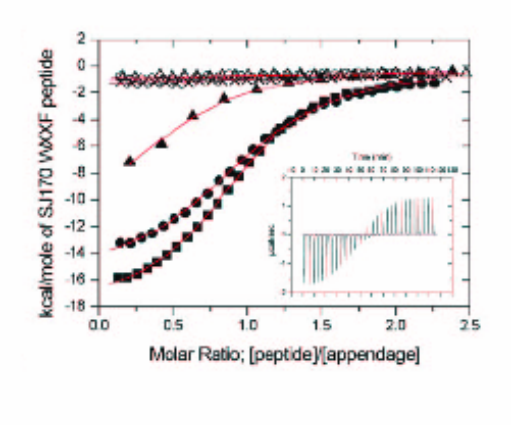


Figure 3.4: ITC measurements of the SJ170 WXXF peptide to AP-2 α appendage.
ITC measurements of the binding of SJ170 WXXF peptide to wild-type and mutant AP-2 α-appendages. Traces are shown after subtraction of data from injection of peptide into buffer blank. The inset shows raw data from SJ170 WXXF peptide (1 mM) injected into wild-type α-appendage (100 μM) in 10-mL aliquots.

α-appendage	SJ170 peptide	symbol	K_d(μM)
wild-type	KGWVTFEE	■	10.7 ± 0.3
R905A	KGWVTFEE	▲	9 ± 1
R916A	KGWVTFEE	●	13.8 ± 0.5
wild-type	KGAVTFEE	△	N.D.*
G725N	KGWVTFEE	x	N.D.*
Q782A	KGWVTFEE	○	N.D.*

* No binding detected within a limit of ~250 μM

Figure 3.5: K_d values from the ITC experiments.
Dissociation equilibrium constants (K_d's) derived from the ITC experiments.

Table 3.1: Effect of sandwich domain point mutations on binding of the α_c appendage to the SJ170 WXX(FW)X(DE) motif and brain binding partners.

Protein	Relative binding to SJ170M1[a]	Relative binding in brain cytosol pull-down			
		NECAP-1	Epsin 1	AP180	Amphiphysin
GST	−	−	−	−	−
GST-wild-type α_C appendage	++++	++++	++++	++++	++++
GST-α_C appendage mutant R707S	++++	ND[b]	ND	ND	ND
GST-α_C appendage mutant N712Y	+++	ND	ND	ND	ND
GST-α_C appendage mutant Q723A	ND	−	++++	++++	++++
GST-α_C appendage mutant G725N	+	−	++++	++++	++++
GST-α_C appendage mutant K727A	++++	+/−	++++	++++	++++
GST-α_C appendage mutant R731A	++++	ND	ND	ND	ND
GST-α_C appendage mutant F740D	−	−	++++	++++	++++
GST-α_C appendage mutant Q782A	−	−	++++	++++	++++

[a] Semiquantitative indication of W*XX*(FW)*X*(DE) sequence binding to the various AP-2 α_C appendages relative to the wild-type protein (experiments performed as described for Fig. 2*D*).
[b] Not determined.

= 9 ± 1 μM) and FXDXF (R916A, K_d= 13.8 ± 0.5 μM) motif binding displayed minor differences in affinity for the WXX[FW]X[DE] motif peptide. However, mutations in the Trp-preferential binding site (G725N and Q782A) completely preclude detectable binding of the peptide. These results also reflect the strict requirement for the first Trp in the WXX[FW]X[DE] motif [82] as substitution of the proximal Trp in the peptide for Ala completely abolishes measurable peptide binding (Fig. 3.5).

In pull-down assays with brain cytosol, Q723A, G725N, F740D, and Q782A mutations each completely abolish the association of NECAP 1 (which has a single carboxy-terminal ^{272}WVQF sequence [202]) with the immobilized α_C appendage without perturbing epsin, AP180 or amphiphysin binding (Fig. 3.1A, 3.1B, and Table 3.1). In this assay, a K727A α appendage mutation perturbs soluble NECAP 1 binding less completely (Table 3.1). With PC12 cell extracts, the Q782A mutation also significantly blunts SJ170 binding (Fig. 3.1A); we attribute the remaining SJ170 binding to the intact FXDXF, DP[FW] (and WXX[FW]X[DE], see below) motifs binding to the unaltered platform subdomain site. The apparently normal binding of cytosolic SJ170 to GST-α_C appendage (G725N) mutant may be related to the relatively weaker effect of this mutation on SJ170 binding (Fig. 3.3). Thus, five of the seven residues that generate the sandwich subdomain Trp-selection region are variably important for WXX[FW]X[DE] motif engagement.

3.3.2 A Phylogenetically-Conserved α-Subunit Specific Binding Site

The sandwich subdomain interaction surface appears to have been conserved on the α appendage through evolution. Sequence alignments reveal that of the seven key residues that generate the

Trp binding site (Fig. 3.2B), three (Gln723, Gly725 and Gln728) are invariant from *Schizosaccharomyces pombe* to mammals and are also conserved in *Arabidopsis thaliana* (Fig. 3.6). The remaining residues (Gly714, Val715, Lys727 and Phe740) are all conservatively substituted (Fig. 3.6). In fact, surface exposed side chain conservation is only evident on the A'/A/B/E sheet of the appendage sandwich as the C/D/F/G strand-containing opposite side displays virtually no surface phylogenetic conservation (Fig. 3.6) [69]. The strong sequence preservation argues strongly that the interaction surface on the sandwich subdomain plays a physiologically important role in AP-2 function.

The AP-2 β2-subunit appendage, which is structurally and functionally analogous to the α appendage [70], does not display a homologous interaction surface at the equivalent position on the sandwich subdomain. Indeed, the WXX[FW]X[DE] sequence displays a very high selectivity for the α appendage over the β2 (Fig. 3.7, SJ170 and NECAP 1 binding) [82, 135, 202]. The AP-1 γ-subunit appendage and the structurally homologous GGA protein GAE domain [68, 204] bind to a D[FW]GXØ motif [205, 206] superficially similar to the AP-2-binding WXX[FW]X[DE]motif. In fact, the AP-1 γ appendage binds a ^{382}WNSF sequence present in the hinge of GGA1 [207]. While the γ appendage/GAE domain is composed solely of a β-sandwich domain and lacks the platform subdomain characteristic of the bilobal α and β-subunit appendages, the binding site for the D[FW]GXØ motif is located on the opposite side of the β-sandwich from the WXX[FW]X[DE] interaction surface and also lacks the strict specificity for Trp at the 0 position [208-212]. Together, these data argue that the WXX[FW]X[DE] binding site on the α appendage sandwich plays a unique role in AP-2 activity.

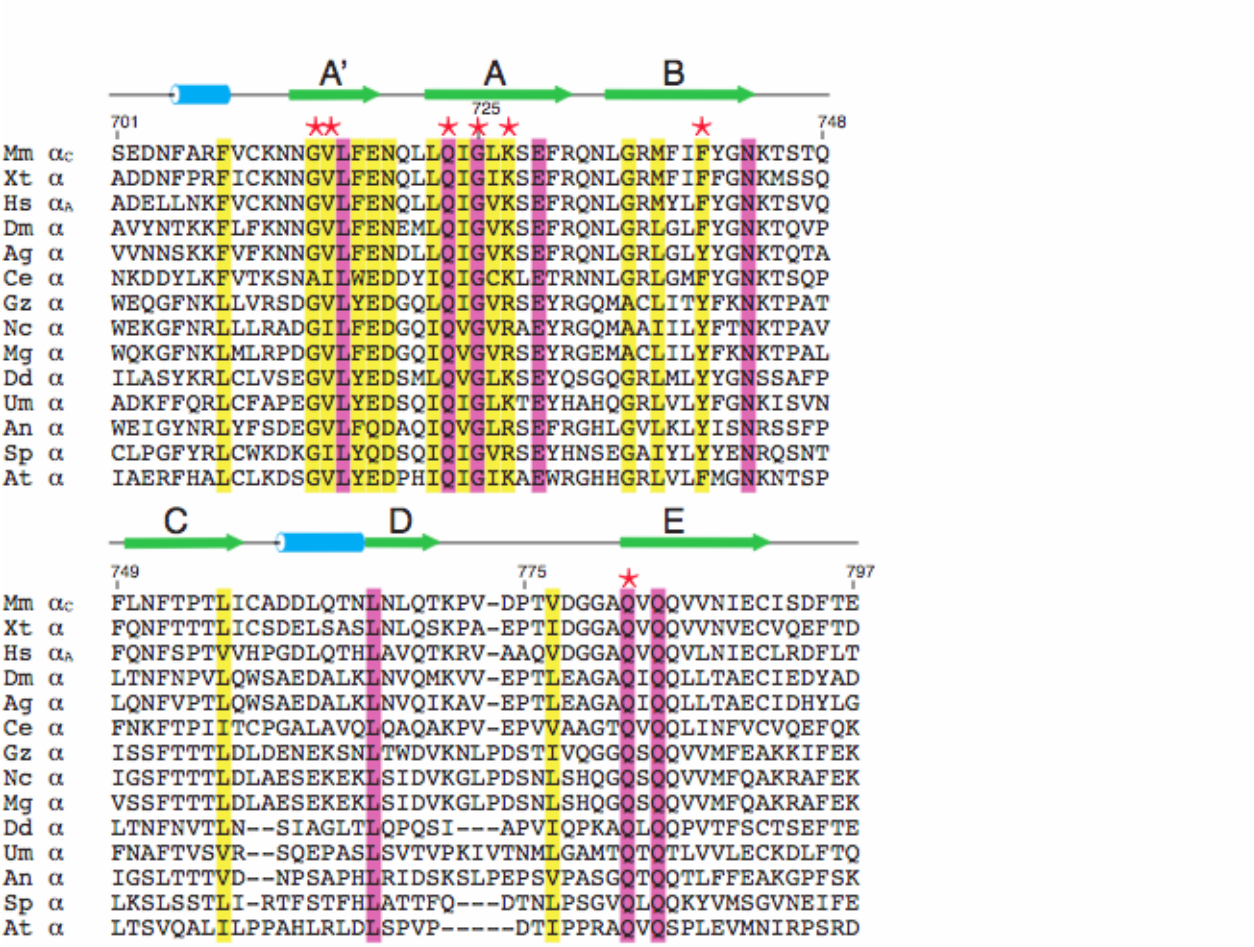


Figure 3.6: Evolutionary preservation of the binding site upon the α appendage sandwich.
Primary sequence alignment of a subunits from *Mus musculus* (Mm; accession number P17427), *Xenopus tropicalis* (Xt; AAH67918), *Homo sapiens* (Hs; AAL11040), *Drosophila melanogaster* (Dm; NP_476819), *Anopheles gambiae* (Ag; XP_310153), *Caenorhabditis elegans* (Ce; NP_509572), *Dictyostelium discoideum* (Dd; AAO51059), the fungi *Gibberella zeae* (Gz; XP_381000), *Neurospora crassa* (Nc; XP_322698), *Magnaporthe grisea* (Mg; EAA53743), *Ustilago maydis* (Um; EAK81870), *Aspergillus nidulans* (An; EAA61662), *Schizosaccharomyces pombe* (Sp; NP_595595), and *Arabidopsis thaliana* (At; NP_197670). Residue numbering and secondary structure elements from the mouse α_C appendage structure are indicated above while invariant side chains are highlighted in pink and chemically conserved substitutions in yellow. Residues that contribute to the WXX[FW]X[DE] interaction surface are indicated with a red asterisk. Notice little phylogenetic conservation on the strands (C and D) that form part of the β-sheet opposite to the A'/A/B and E sheet containing the WXX[FW]X[DE] binding site.

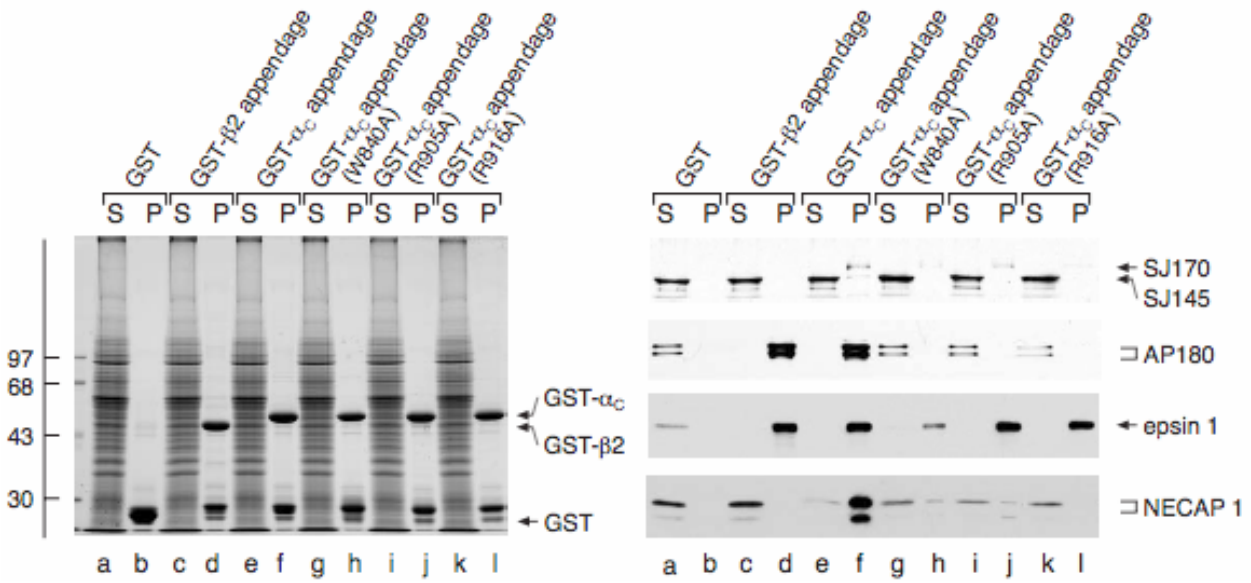


Figure 3.7: α appendage platform mutations affect WXXF-containing binding partners.
Approximately 50 μg of either GST (lane a and b), GST-β2 appendage (lane c and d), GST-α_C appendage (lane e and f) or the GST-α_C appendage point mutants W840A (lane g and h), R905A (lane i and j), or R916A (lane k and l) immobilized on GSH-Sepharose was incubated with PC12 cell lysate. After centrifugation, aliquots corresponding to 1/60 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed with the anti-synaptojanin 1 antibody AR/1, or an anti-AP180 mAb, or anti-epsin 1 or anti-NECAP 1 antibodies.

3.3.3 Multisite α Appendage Engagement

Human SJ170 has an alternatively-spliced carboxy-terminal extension containing a WXX[FW]X[DE], a FXDXF, and two DPF motifs (see Fig. 3.16) [72, 82, 213]. In earlier work, we showed that the WXX[FW]X[DE] and FXDXF sequences together promote optimal α_C appendage engagement [82]. Three lines of evidence suggest that multiple WXX[FW]X[DE] motifs, as found in the endocytic protein stonin 2 [134, 135, 214], might enhance α appendage binding similarly. First, while addition of a WXX[FW]X[DE] peptide completely abrogates binding of the NECAP 1 in PC12 cell extracts to immobilized GST-α_C appendage, the peptide also weakly inhibits binding of AP180 (Fig. 3.1A, lane f), which does not contain a WXX[FW]X[DE] motif. By contrast, a G725N or Q782A mutation prevents NECAP binding but has no effect on AP180 association with the mutated α_C appendage (Fig. 3.1A, lane h and j). Similar experiments using brain cytosol also show that the stonin 2 WXX[FW]X[DE] peptide, while abolishing NECAP binding, diminishes the association of AP180 and amphiphysin I and II with immobilized α_C appendage (Fig. 3.1B lane f compared to lane d). One interpretation of these results is that the WXX[FW]X[DE] motif may also bind weakly to the major interaction surface on the platform subdomain of the α_C appendage, although the ITC data with the α_C (Q782A) mutant indicate that the affinity of this interaction is poor.

Second, the binding of cytosolic SJ170 and NECAP 1 to the GST-α_C appendage is perturbed not only by sandwich domain mutants (G725N, F740D, Q782A, Fig. 3.1A, 3.1B, and Table 3.1) but also by the platform domain mutants W840A, R916A, and somewhat less by R905A (Fig. 3.7). Interestingly, the reduced recovery of NECAP 1 in the supernatant fractions

(compare lanes g, i, and k with lane a and c) together with the trace levels of NECAP 1 in the GST-α_C appendage pellet fractions (compare lanes h, j, and l with lane f) suggests that the platform mutations alter the off-rate of wild type NECAP 1, and the bound pool of protein is lost during the washing steps. The position of these modulatory side chains (Trp840, Arg905, Arg916) relative to the WXX[FW]X[DE] binding site on the sandwich subdomain (Fig. 3.2A), the large buried interfacial surface area between the sandwich and platform subdomains, and rigidity of the two subdomains relative to one another [69] makes it unlikely, in our view, that these platform mutations propagate a conformational effect to the sandwich subdomain.

Third, although truncation of the amino-terminal region of stonin 2 containing three WXX[FW]X[DE] motifs (Fig. 3.8) to contain two motifs has little effect on AP-2 binding, a GST-stonin 2 fusion containing only the first WXX[FW]X[DE] sequence binds AP-2 very weakly (Fig. 3.9). Since the GST-stonin 2 is present in large excess in these experiments, the data are consistent with the idea that tandemly arrayed WXX[FW]X[DE] motifs cooperate to increase the apparent affinity for the AP-2 α_C appendage. Similar results are obtained if individual WXX[FW]X[DE] motifs are inactivated singly or in combination in the context of a GST-stonin 2 (1-247) fusion protein (Fig. 3.10). Again, a single WXX[FW]X[DE] sequence shows a marked reduction in AP-2 binding that, given the selectivity of the motif for the α appendage, appears inconsistent with a single binding site upon the appendage.

To extend these observations, we first established whether the amino-terminal portion of stonin 2, which contains only WXX[FW]X[DE] motifs, can inhibit partner protein binding to the α_C appendage platform. Supplementing brain cytosol with 20 μM stonin 2 (1-247) largely prevents the association of AP180 and amphiphysin I and II with immobilized GST-α_C appendage (Fig. 3.11, lane f compared with lane d) without perturbing epsin 1 binding. This

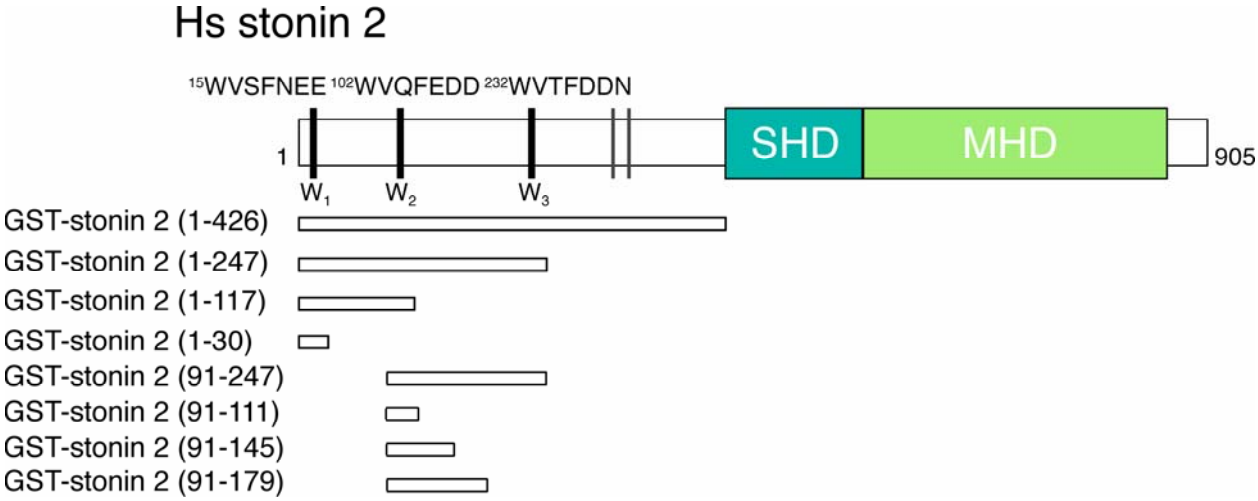


Figure 3.8: Schematic representation of stonin 2 and the constructs used in this study.
Schematic representation of the organization of human (Hs) stonin 2 and the relative location of the deletion constructs used in this study. SHD, stonin homology domain; MHD, μ subunit homology domain.

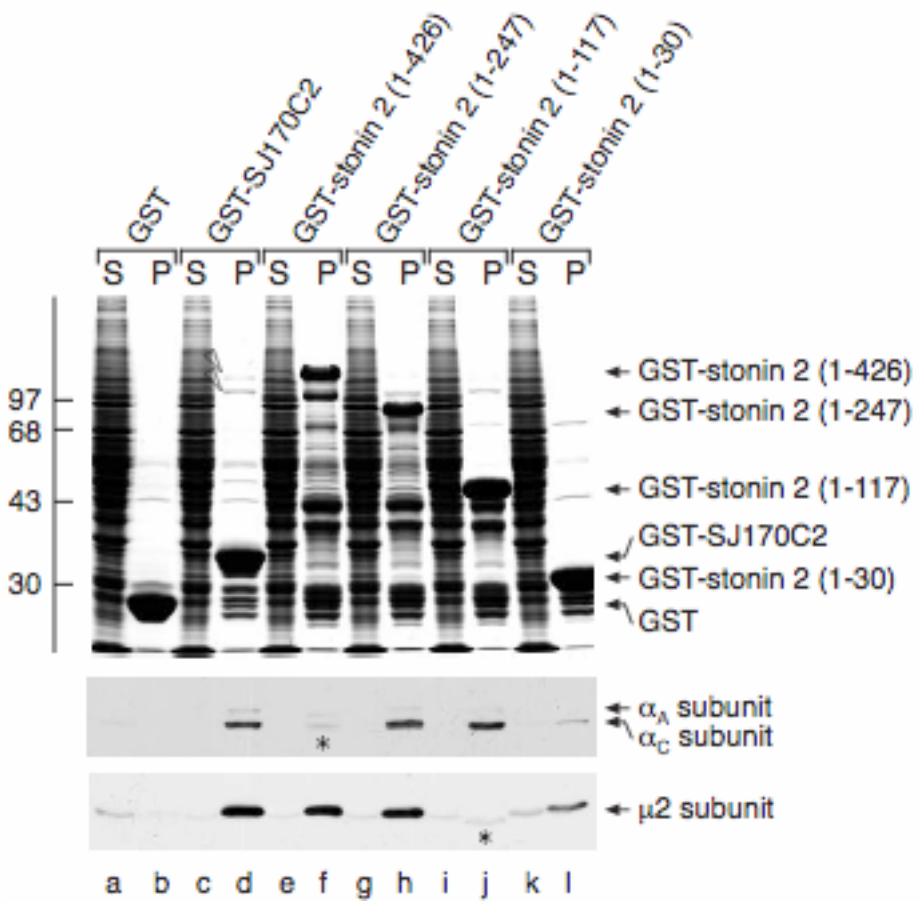


Figure 3.9: Binding of the AP-2 adaptor to the stonin 2 WXXF repeats in deletion constructs.
Approximately 100 μg of either GST (lane a and b), GST-SJ170C2 (residues 1454-1530) (lane c and d), GST-stonin 2 (1-426) (lane e and f), (1-247) (lane g and h), (1-117) (lane i and j), or (1-30) (lane k and l) immobilized on GSH-Sepharose was incubated with rat brain cytosol. After centrifugation, aliquots corresponding to 1/40 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed with the anti-AP-2 α subunit mAb 100/2 or anti-μ2 subunit serum. Arrowheads indicate the position of the large α and β2 subunits of the AP-2 complex, while the asterisks indicate where protein transfer has be reduced by the large amount of GST-fusion protein comigrating at that position.

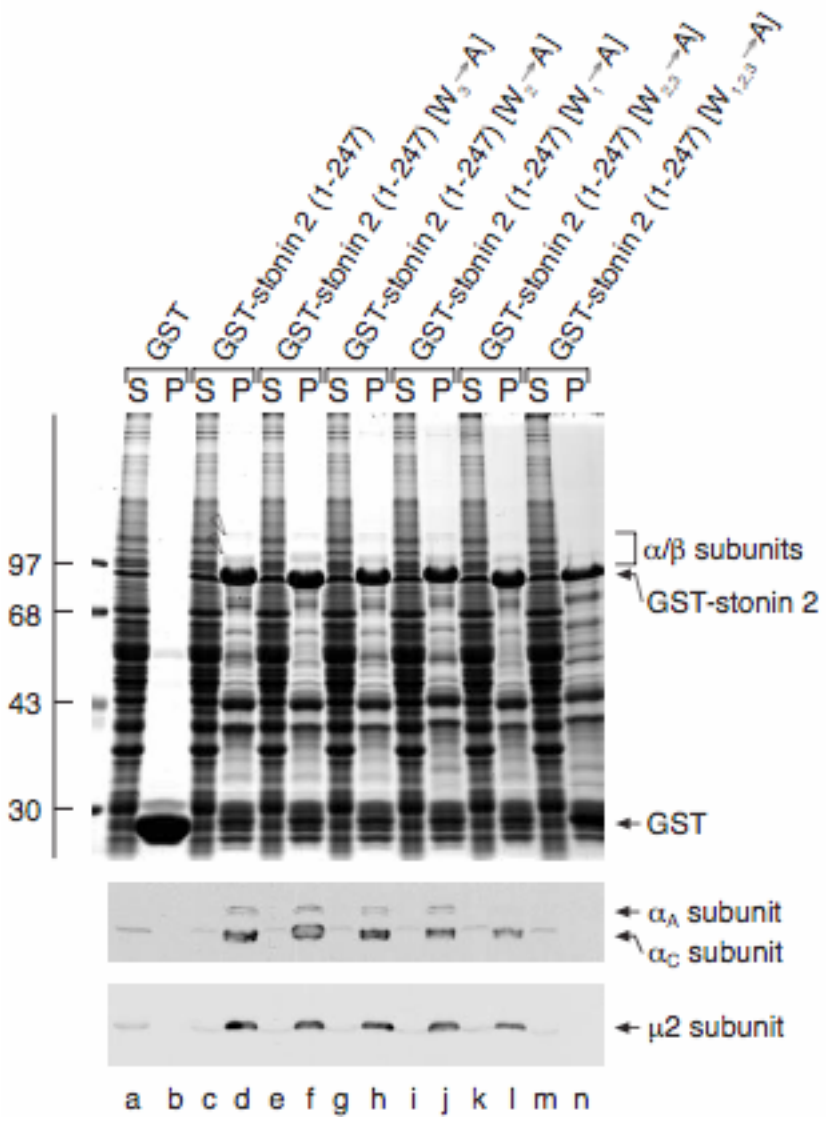


Figure 3.10: Binding of the AP-2 adaptor to the stonin 2 WXXF repeats in mutant constructs.
Approximately 100 μg of either GST (lane a and b), GST-stonin 2 (1-247) (lane c and d), (1-247) [W_3→A] (lane e and f), (1-247) [W_2→A] (lane g and h), (1-247) [W_1→A] (lane i and j), (1-247) [$W_{2,3}$→A] (lane k and l), or (1-247) [$W_{1,2,3}$→A] (lane m and n) immobilized on GSH-Sepharose was incubated with rat brain cytosol. After centrifugation, aliquots corresponding to 1/40 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed with the anti-AP-2 α subunit mAb 100/2 or anti-μ2 subunit serum. Arrowheads indicate the position of the large α and β2 subunits of the AP-2 complex.

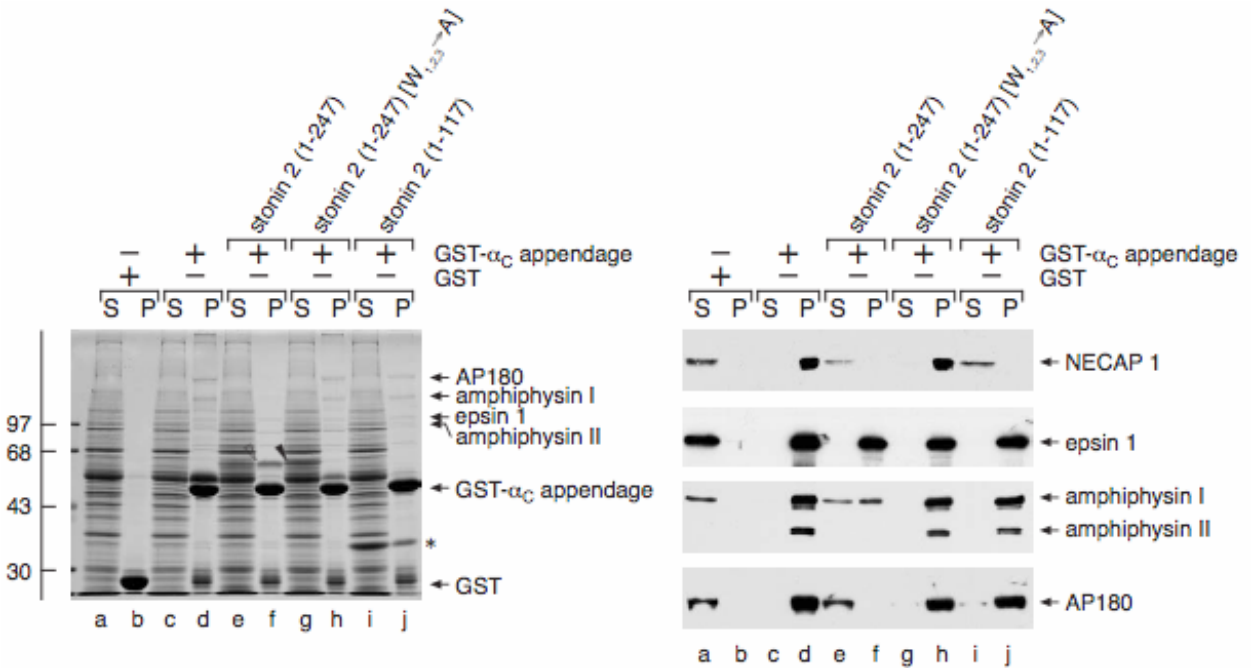


Figure 3.11: Residues adjacent to the WXXF motifs can contribute to platform binding once bound to the sandwich site.

Approximately 50 μg of immobilized GST (lane a and b) or GST-α_C appendage (lane c-k) were incubated with rat brain cytosol alone (lane a-d) or with cytosol supplemented with 20 μM stonin 2 (residues 1-247; lane e and f), 20 μM stonin 2 (1-247) [$W_{1,2,3} \rightarrow A$] (lane g and h), or 20 μM stonin 2 (1-117) (lane i and j). After centrifugation, aliquots of approximately 1/40 of each supernatant (S) and 1/6 of each washed pellet (P) were resolved by SDS/PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed anti-NECAP 1 or epsin 1 antibodies, or an anti-amphiphysin or anti-AP180 mAb. The open arrowhead indicates the added wild type stonin 2 (1-247) polypeptide, the closed arrowhead the stonin 2 (1-247) [$W_{1,2,3} \rightarrow A$] and the asterisk the stonin 2 (1-117) polypeptide.

difference is due to avidity/chelate effects since epsin 1 has eight tandemly arrayed DPW motifs while amphiphysin, for example, has only one DPF and one FXDXF motif. In these experiments, a portion of the added stonin 2 fragment now associates with the sedimented appendage (open arrowhead) and, as expected, NECAP 1 binding is completely abolished. The capability of the stonin 2 (1-247) fragment to inhibit when bound at only substoichiometric levels suggests that multiple α_C appendages can be engaged by a single stonin 2 molecule. An equivalent concentration of a stonin 2 (1-247) [$W_{1,2,3}\rightarrow A$] mutant fails to bind to the GST-α_C appendage (arrowhead), and has no effect on the association of either NECAP 1 or AP180 and amphiphysin with GST-α_C appendage. Surprisingly, addition of a 5-fold molar excess of a smaller segment of stonin 2 (residues 1-117), containing only two WXX[FW]X[DE] repeats, inhibits AP180 binding very weakly (compare lane j to lane d) without perturbing amphiphysin binding. Nevertheless, the stonin 2 (1-117) binds to the immobilized appendage (asterisk) abolishing the NECAP 1 interaction. These results suggest that other residues adjacent to the WXX[FW]X[DE] motifs can also contribute to binding to the platform once the polypeptide is bound to the sandwich.

To dissect the mode of inhibition further, we analyzed the effect of inactivating the sandwich site on the inhibitory action of the stonin 2 (1-247) and SJ170C2 (residues 1454-1530) protein segments. When added to cytosol, each segment binds to the GST-α_C appendage preventing binding of AP180 and amphiphysin as well as NECAP 1 (Fig. 3.12, lanes f and h compared to lane d). Similar experiments with immobilized GST-α_C appendage (Q782A) reveal that an intact sandwich site is necessary for optimal inhibition by both the stonin 2 (1-247) and SJ170C2 proteins (lanes l and n compared to lanes d and j). Our interpretation of these results is that the WXX[FW]X[DE] motif, by binding to the sandwich site, enhances the affinity of other interaction sequences located within these essentially unstructured protein regions for the

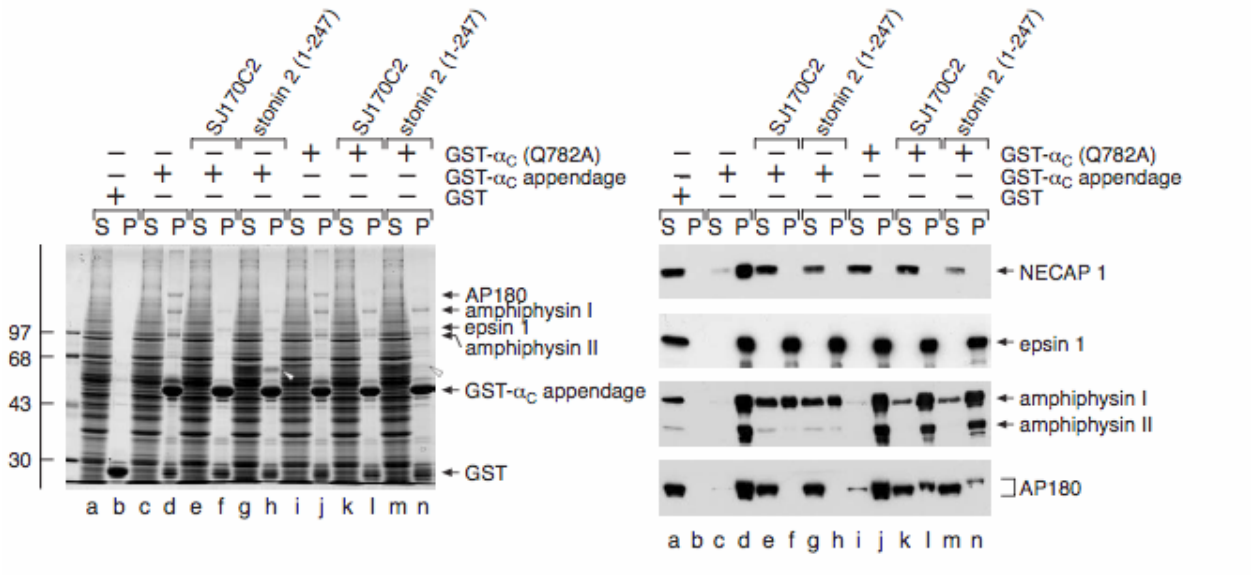


Figure 3.12: The WXXF motif enhances the affinity of other sequences for the platform site.
Approximately 50 μg of immobilized GST (lane a and b), GST-α_C appendage (lane c-h), GST-α_C appendage (Q782A) (lane i-n) were incubated with rat brain cytosol alone (lane a-d) or with cytosol supplemented with 20 μM SJ170C2 (residues 1-247) (lane e, f and k, l), or 20 μM stonin 2 (1-247) (lane g, h and m, n). After centrifugation, aliquots of approximately 1/40 of each supernatant (S) and 1/6 of each washed pellet (P) were resolved by SDS/PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed anti-NECAP 1 or epsin 1 antibodies, or an anti-amphiphysin or anti-AP180 mAb. The open arrowheads indicate the position of the added wild type stonin 2 (1-247) polypeptide.

platform site. AP180 binding to GST-α_C appendage (Q782A) is inhibited by these protein fragments but we believe this is because AP180 has the lowest apparent affinity for the α_C appendage [69] and freely soluble WXX[FW]X[DE] motifs can perturb this interaction (Fig. 3.1).

For the SJ170C2 protein segment, which contains one FXDXF and one WXX[FW]X[DE] motif, the concerted action of both motifs in α_C appendage binding is clearly seen upon mutagenic inactivation of either motif separately (Fig. 3.13). Importantly, in this case, altering the FXDXF motif to AXAXF (SJ170C2 [FD→A]) reverses the competitive effect of the inhibitor on AP180 and amphiphysin binding but has no effect on NECAP 1 inhibition (lane j compared to lane h). By contrast, substituting the WXX[FW]X[DE] motif with AXX[FW]X[DE] (SJ170C2 [W→A]) still reverses the inhibitory effect of the fragment on AP180 and amphiphysin binding but relieves the inhibition of NECAP 1 binding (lane l compared to lanes h and j). Together, these data show that the capability of the SJ170C2 portion to inhibit α_C appendage interactions requires both interaction motifs and both α_C appendage-binding sites.

Combinations of WXX[FW]X[DE] and DP[FW] motifs are also found tandemly arrayed in invertebrate endocytic components. In *D. melanogaster* Numb-associated kinase (NAK; NP_477165), an Ark1/Prk1 family Ser/Thr protein kinase related to mammalian AAK1 and GAK/auxilin 2 [215], a ^{586}WNPFEEE sequence is positioned between two DPF triplets in a central segment predicted to be unstructured. Related sequences are present in presumptive *Anopheles gambiae* (WNPFGDP; XP_321932) and *Apis mellifera* (WNPFEDV; BI515476) orthologues with adjacent DPF triplets. The WNPFX[DE] sequence is homologous to a major ^{695}WNPFDD AP-2 interaction motif in human AAK1 [82] and conforms to the general consensus WXX[FW]X[DE] [82]. In a *C. elegans* protein that is a possible stonin 2 orthologue

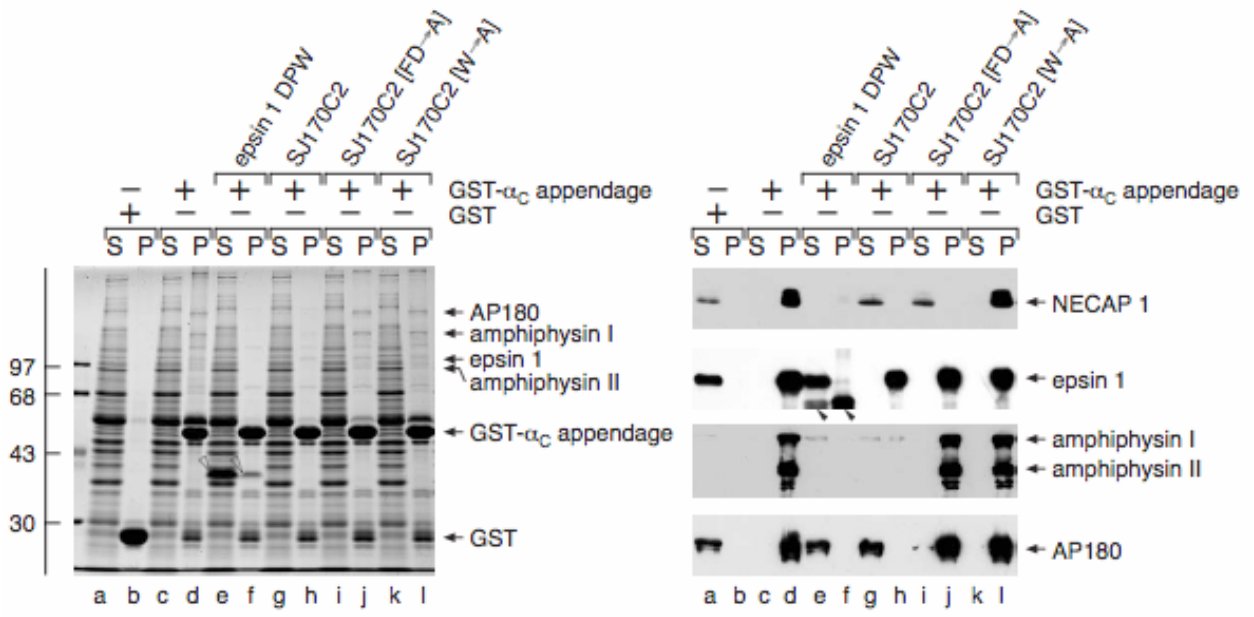


Figure 3.13: The inhibitory ability of SJ170C2 requires both the FXDXF and WXXF motifs.
Approximately 50 μg of immobilized GST (lane a and b) or GST-α_C appendage (lane c-l) were incubated with rat brain cytosol alone (lane a-d) or with cytosol supplemented with 20 μM epsin 1 DPW domain (residues 229-407; lane e and f), 20 μM SJ170C2 fragment (lane g and h), 20 μM SJ170C2 (FD→A) (lane i and j), or 20 μM SJ170C2 (W→A) (lane k and l). After centrifugation, aliquots of approximately 1/40 of each supernatant (S) and 1/6 of each washed pellet (P) were resolved by SDS/PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed anti-NECAP 1 or epsin 1 antibodies, or an anti-amphiphysin or anti-AP180 mAb. The open arrowheads indicate the position of the added epsin DPW (229-407) polypeptide, while the closed arrowheads indicate immunological detection of the added epsin DPW competitor polypeptide.

(APT-10; NP_505566), a potential sandwich binding sequence WADFETS lies between one proximal and two distal DPF repeats. In all these proteins, the individual motifs are separated by al least 15 amino acids, providing ~50 Å of flexible linker polypeptide. Although the activity of these sequences must be confirmed experimentally, the data are consistent with the phylogenetic conservation of the WXX[FW]X[DE] binding surface on the α appendage sandwich subdomain (Figure 3.2A, 3.2B, and 3.6).

Next, we analyzed the functional effect of reducing the number of WXX[FW]X[DE] repeats within the full-length stonin 2 protein on AP-2 localization and transferrin uptake in transiently transfected cells. Others have shown that overexpression of stonin 2 in either HeLa or COS7 cells disrupts the intracellular distribution of the AP-2 adaptor and inhibits transferrin, LDL and EGF internalization [135, 214]. We also find that overexpression of GFP-tagged stonin 2 (1-905) in HeLa cells perturbs AP-2 (Fig. 3.14A). In GFP-stonin 2-expressing cells, there is clearly a more prominent pool of cytosolic AP-2, seen as a diffuse haze compared to the non-transfected cells. The GFP-stonin 2 transfected cells also have fewer punctate AP-2 structures compared to adjacent untransfected cells and, generally, the fluorescence intensity of the remaining AP-2 puncta is reduced in the overexpressing cells. Similar results are obtained upon overexpression of AAK1 in HeLa cells [216]. Overexpression of GFP-stonin 2 (1-905) [$W_{2,3}\rightarrow A$], with only one functional WXX[FW]X[DE] motif, almost completely reverses the effect on AP-2 however (Fig. 3.14B). Likewise, overexpression of wild-type GFP-stonin 2 inhibits the uptake of biotin-transferrin and accumulation in juxtanuclear endosomes (Fig. 3.14C) but the GFP-stonin 2 (1-905) [$W_{2,3}\rightarrow A$] protein does not (Fig. 3.14D). Taken altogether, our data show that the sandwich site is a functional interaction surface upon the α_C appendage that

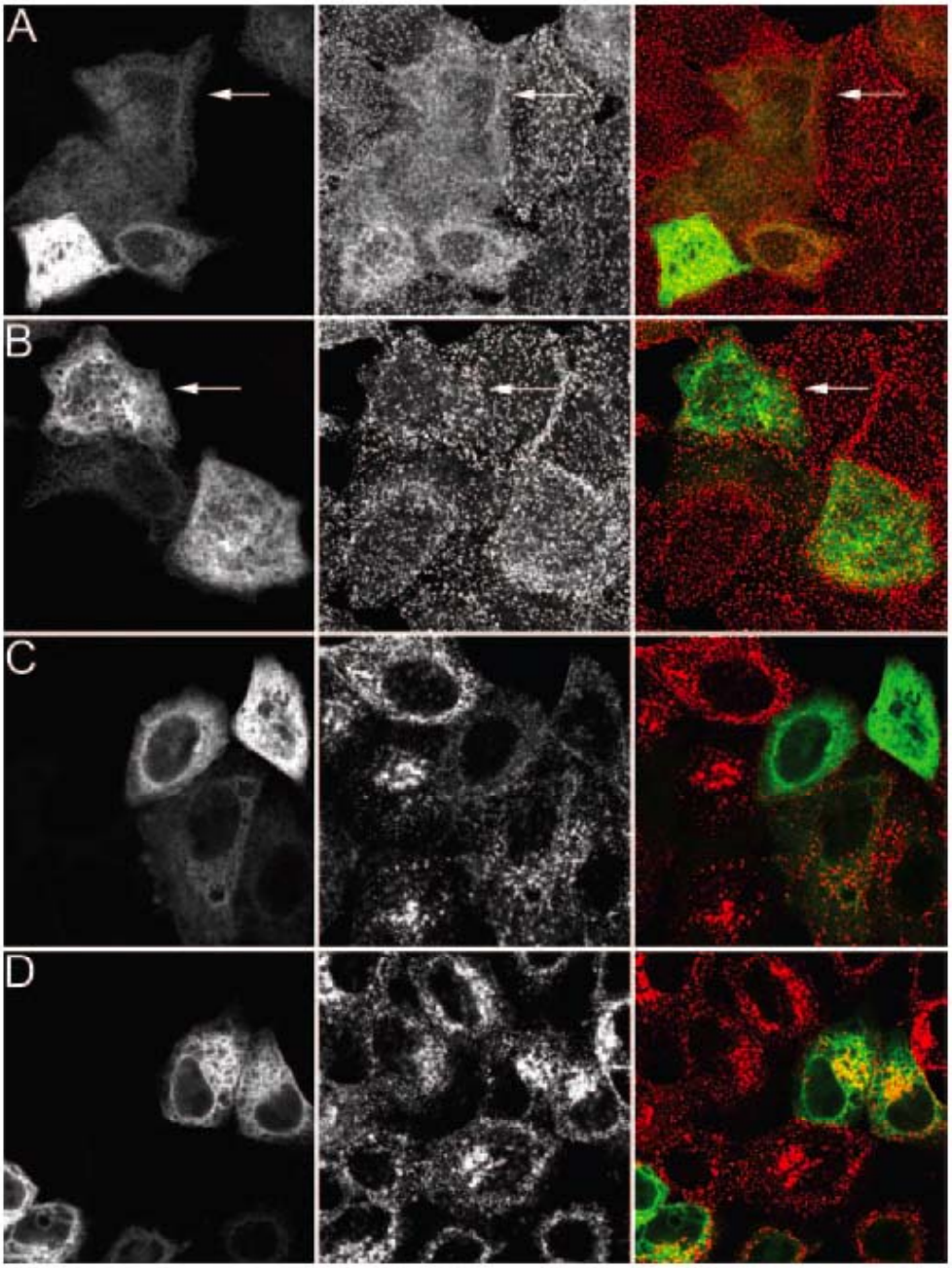


Figure 3.14: Inactivation of two of the three WXXF motifs in stonin 2 prevents overexpression-induced AP-2 reorganization and endocytosis defects.
HeLa cells transiently transfected with either GFP-stonin 2 (1-905) (A and C; green) or GFP-stonin 2 (1-905) [$W_{2,3}\rightarrow A$] (B and D; green) were fixed and stained with the anti-AP-2 α subunit mAb AP.6 (A and B; red) or pulsed with 25 μg/ml transferrin for 15 min at 37°C before fixation (C and D; red). Transfected cells expressing roughly comparable levels of GFP-stonin are indicated by the arrows.

expands the number of engagement modes to permit proteins with tandemly arrayed interaction motifs to regulate the occupancy of the platform.

3.3.4 Motif Arrays Govern Hierarchical Recruitment

Different apparent affinities of soluble α_C appendage binding partners in brain or PC12 cell extracts can be seen in titration experiments (Figure 3.15). In agreement with our competition studies, intact epsin 1 displays the highest affinity for the α appendage while AP180 and NECAP 1 have relatively low apparent affinities (Figure 3.15). In general, there is a good correlation between the presence of multiple interaction motifs and/or the extent of motif repetition (Fig. 3.16) and the observed apparent affinity for immobilized α_C appendage. Importantly, SJ170 has a high affinity for the appendage, and the difference in binding between SJ170 and the short, neuronal-specific isoform of synaptojanin 1, SJ145, is clear. SJ145, which contains only a single WXX[FW]X[DE] motif, binds GST-α_C appendage with an affinity roughly comparable to NECAP 1. A striking finding is that several of the alternate adaptors, including epsin 1, Dab2 and HIP1, that expand the sorting repertoire of clathrin-coated vesicles that form at the plasma membrane have high apparent affinities for the GST-α_C appendage.

In no instance do we observe effective displacement of epsin 1 from immobilized α_C appendage, irrespective of the interaction motifs present in the competitor protein (Fig. 3.1 and 3.7). This indicates that, unlike dynamin and actin [217], epsin might not display dramatic temporal fluctuations during clathrin coat assembly. At steady state, there is a high degree of colocalization of endogenous epsin 1 with clathrin or AP-2 [118] demonstrating that epsin 1 populates the majority of clathrin coated structures located at the cell surface. Immunogold

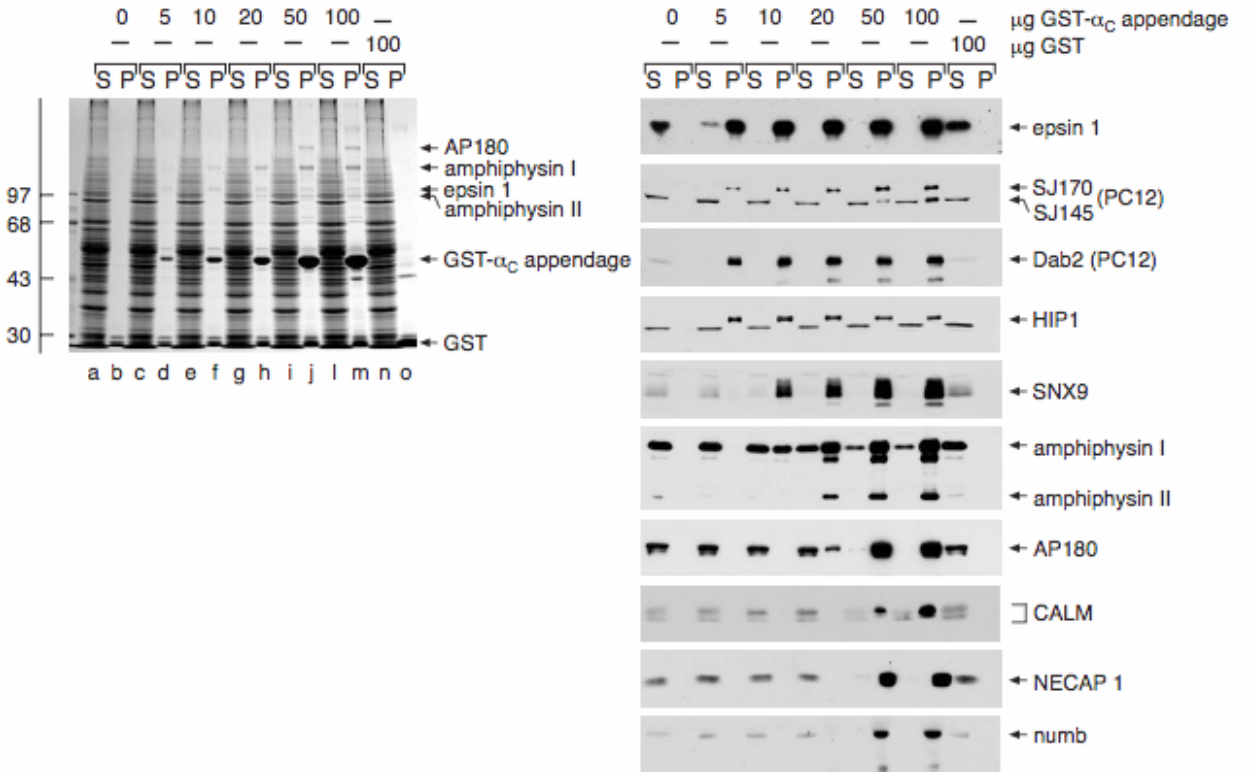


Figure 3.15: A hierarchical set of α appendage binding-partners.
Immobilized GST or GST-α_C appendage (0-100 µg) was incubated with either rat brain or PC12 cell extracts. Aliquots of approximately 1/40 of each supernatant (S) and 1/6 of each washed pellet (P) were resolved by SDS/PAGE and either stained or transferred to nitrocellulose. Portions of the blots were probed anti-epsin 1, -synaptojanin 1, -Dab2, -HIP1, -SNX9, -amphiphysin, -AP180, CALM, NECAP 1 or -numb antibodies.

analysis of the distribution of endogenous epsin 1 at the ventral surface of disrupted NRK or PC12 cells using freeze-etch EM shows that the protein is almost exclusively present within regions of assembled polygonal clathrin lattice (Fig. 3.17B and C). Antibodies against the AP-2 α subunit and epsin 1 reveal extensive presence of these endocytic proteins within regions of flat clathrin lattice. Significantly, epsin 1 labeling is not only restricted to flat lattices but is also found in rounded structures and deeply invaginated profiles reflecting all stages of clathrin-coated vesicle assembly (Fig. 3.17C, arrows). A very recent report, using our anti-epsin antibodies, also shows that epsin 1 is found in flat clathrin lattices at the cell surface as well as in deeply invaginated clathrin-coated buds in HeLa cells [218]. Together, these results suggest that epsin does not obligatorily exit the assembling clathrin bud prior to the fission event. Indeed, the only accessory factor identified in a recent proteomic analysis of purified brain clathrin-coated vesicles was epsin 1 and epsin is enriched in purified clathrin-coated vesicle preparations [163]. These observations support the proposed action of epsin as an alternate adaptor [48, 147] and again underscore the differential residencies conferred upon endocytic proteins by assemblies of different α_C appendage interaction motifs.

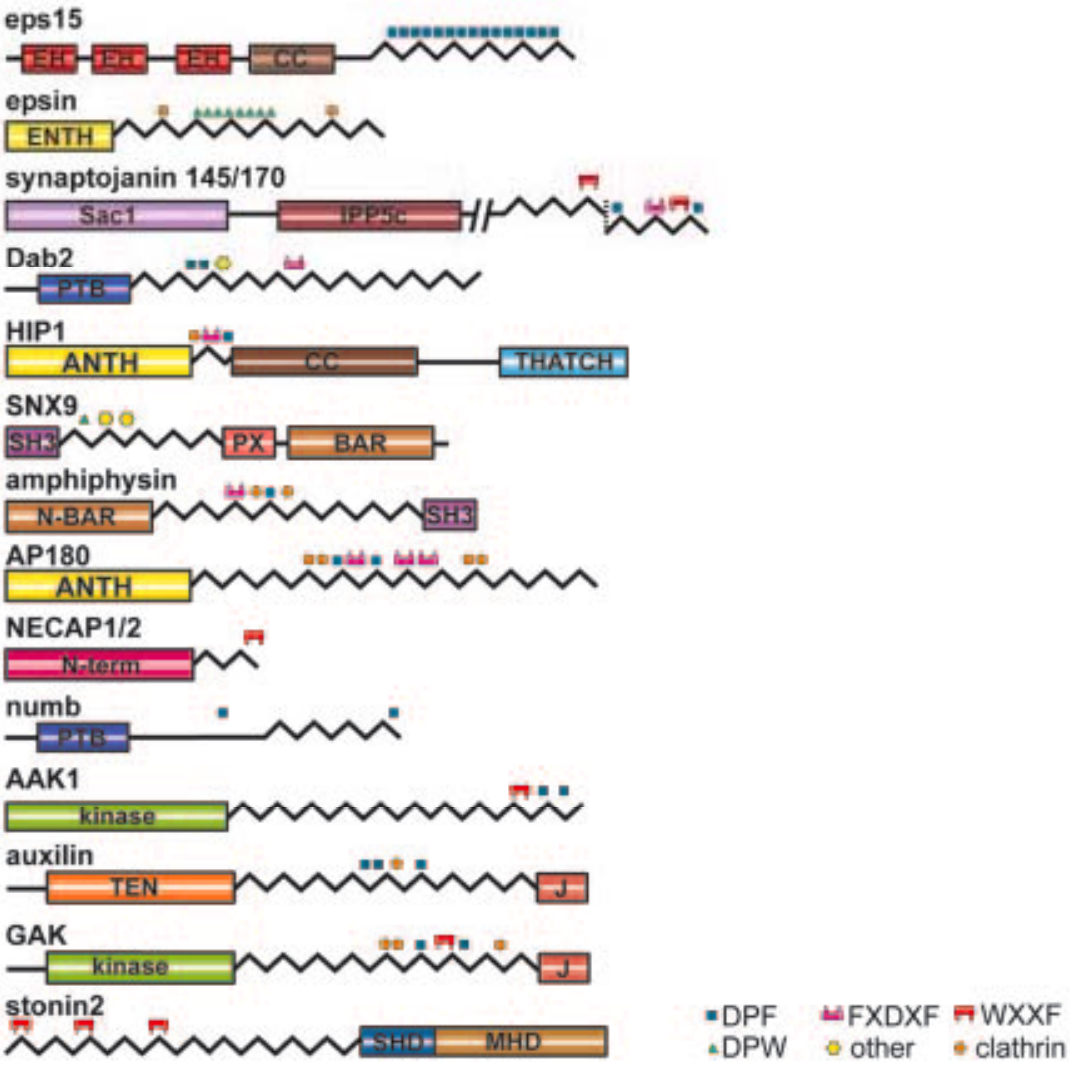


Figure 3.16: Schematic representation of α-appendage binding domains in endocytic accessory proteins.
Regions not corresponding to known domains are symbolized by straight lines (—) while similar regions for which secondary structure cannot be predicted (Jpred2) are indicated by a zig-zag line (〰). Note that α-appendage and clathrin binding motifs occur in regions for which secondary structure cannot be predicted. Domain labels are: ANTH, AP180 N-terminal-homology; BAR, BIN-amphiphysin-RVS; CC, coiled coil; EH, eps15 homology; ENTH, epsin N-terminal-homology; IPP5c, inositol 5-phosphatase catalytic domain; J, DNAJ domain; PTB, phosphotyrosine binding domain; PX, phox domain; Sac1, suppressor of actin 1; SH3, Src homology 3; TEN, tensin homology; THATCH, talin-HIP1/1R actin-tethering C-terminal homology.

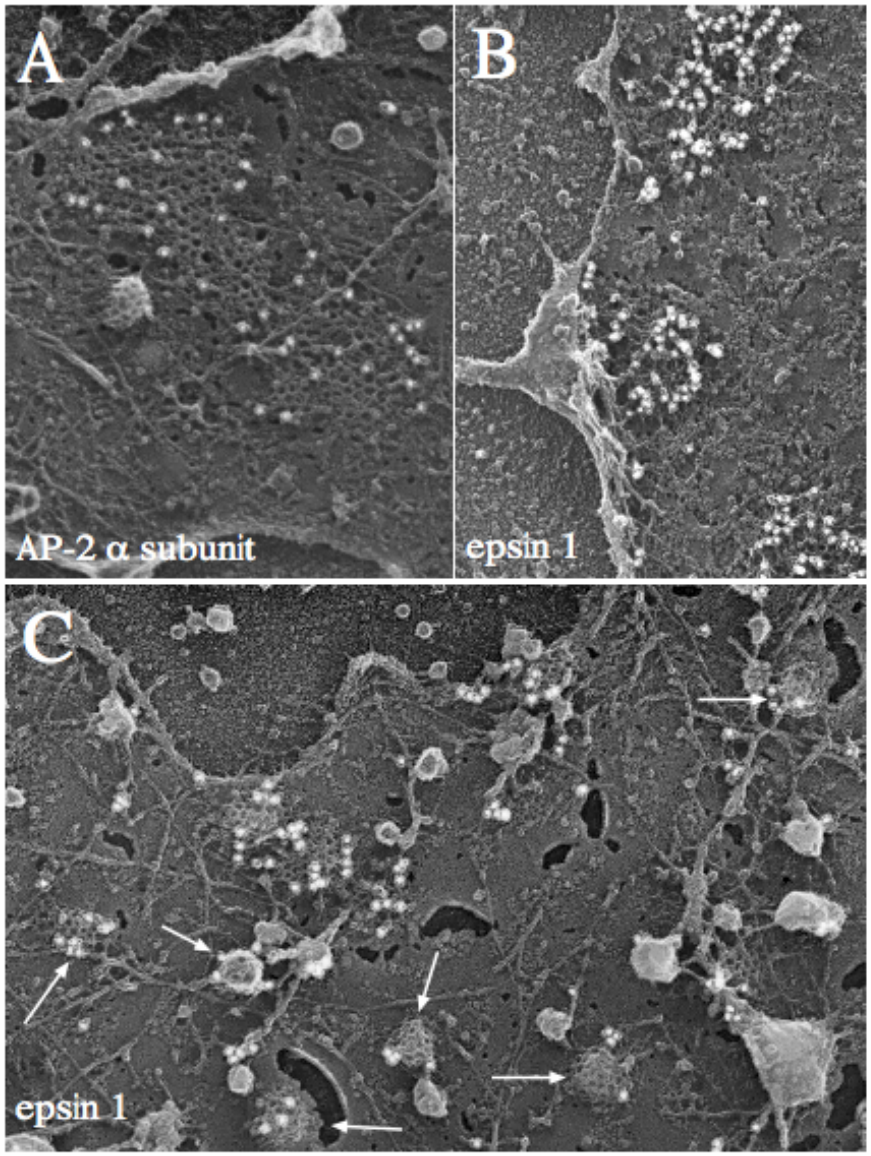


Figure 3.17: Ultrastructural localization of epsin 1 in clathrin-coated structures at the cell surface.
(A-C) Fixed plasma membranes sheets prepared from either NRK (A and B) or PC12 (C) cells labeled with either anti-AP-2 a subunit (A) or anti-epsin 1 antibodies (B and C) and secondary antibodies conjugated to 15 nm colloidal gold particles. Individual gold particles appear as white spheres and representative freeze-etch images show the distribution of endogenous AP-2 and epsin 1. Note extensive labeling for epsin in flat clathrin lattices as well as labeling of rounded structures that reflect the progressive invagination of the lattice to form clathrin-coated vesicles (arrows).

3.4 DISCUSSION

Although the assembly of clathrin-coated vesicles at the plasma membrane requires only a minute or two, the process is characterized by an intricate and complex series of protein-protein interactions. These interactions govern the assembly of polyhedral clathrin lattice, the preferential retention of select cargo molecules, the invagination of the nascent transport intermediate and, finally, scission from the cell surface and uncoating. At least 20 accessory factors, in addition to the clathrin–AP-2–cargo triad, participate in these events [67]. Several of these so-called accessory proteins (AP180, HIP1, epsin 1, Dab2) appear to be *bona fide* alternate adaptors that cooperate with AP-2 during lattice assembly while simultaneously expanding the sorting repertoire of the bud [48]. Others, like amphiphysin, play a more structural role while enzymatic proteins like AAK1, GAK and synaptojanin 1 regulate cargo selection and clathrin coat disassembly [67, 68, 215]. Despite these distinct functions, many of the accessory proteins contact the assembling coat in a similar fashion, by binding to the AP-2 α appendage. A critical deficiency in our understanding of the molecular basis of clathrin-coated vesicle assembly is a lack of a detailed knowledge of the precise chronology and location of the myriad of protein-protein contacts necessary for the successful fabrication and release of a vesicle. We show here that associations with the α appendage utilize two spatially distinct binding surfaces and at least three discrete interaction sequence types. Furthermore, different assemblies of distinct interaction sequences produce proteins with different apparent affinities for AP-2 and generate a hierarchical set of interaction partners.

The measured affinities of the different α appendage binding motifs are in accord with a hierarchical model for binding, a K_d of ~10 μM for the WXX[FW]X[DE] sequence in SJ170

making the sandwich site the highest affinity single interaction. In the alternatively-spliced SJ170 carboxyl terminus, the WXX[FW]X[DE] sequence is the dominant interaction motif, as are the WNPF and WAAW sequences in AAK1 and GAK/auxilin 2, respectively [82]. A single DPF motif has a K_d of ~120 μM [203], and model proteins with one to three DPF triplets bind AP-2 extremely poorly [82, 118]. The FXDXF motif likely has an intermediate affinity as the stonin 2 (1-247) protein, with 3 WXX[FW]X[DE] motifs, effectively competes off amphiphysin and AP180, both with FXDXF sequences. The WNPF and WAAW sequences in AAK1 and GAK/auxilin 2 are atypical WXX[FW]X[DE] motifs that might enable these proteins to bind to both AP-2 and AP-1, through the sandwich domain of the γ subunit appendage, albeit via different interaction surfaces. The γ appendage binds a related WNSF sequence in GGA1 [207] and a bifunctional interaction motif could allow AAK1 to regulate cargo capture by phosphorylation of adaptor μ subunits [57] at different intracellular sites. Similarly, GAK is found in clathrin-coated vesicle preparations [56, 163, 219], binds both the AP-1 γ subunit and the AP-2 α subunit appendages and phosphorylates the μ subunits of these adaptors [219, 220]. The lack of a discernable phenotype after AAK1 silencing by RNAi in HeLa cells [216] also suggests functional redundancy between these Ark1/Prk1 family kinases. However, it is important to note that there is currently no direct evidence for a binary AAK1•γ appendage interaction, and the presence of an acidic residues following the distal Phe (WNPFDD) could interfere with or prevent binding to the γ appendage sandwich site.

Our studies have uncovered two general modes of α_C appendage engagement both characterized by multisite binding. Two fixed binding sites on the appendage and arrays of varied interaction motifs within the binding partners dictate different thresholds for binding to and residency on the α_C appendage. For endocytic proteins like epsin 1 and eps15, tandemly

repeated DP[FW] sequences can promote simultaneous binding to multiple AP-2 molecules by both the α_C and β2 appendages. However, the K_d for these interactions are weak (>100 μM) [203] and, consequently, display interaction half times of only a few seconds which will allow other proteins access to the platform. Yet, the multiplicity of binding motifs counteracts diffusion limited exit by statistically favoring rapid rebinding. This accounts for the high apparent affinity of epsin 1 for the α_C appendage [69]. These motifs, together with embedded clathrin binding sequences, ensure placement of epsin within the lattice throughout the assembly process. A second strategy, typified by SJ170, stonin 2, and possibly AAK1 and GAK [82], is engagement of a spatially distinct binding site on the appendage that, through avidity effects, can promote engagement of other motifs in the platform site. One utility of the spatially distinct WXX[FW]X[DE] binding site on the sandwich subdomain could be that it prevents mutually exclusive interactions by providing a relatively privileged surface for the recruitment of important regulatory components. For AAK1 and GAK, this would ensure that cargo capture by membrane associated AP-2 is not impeded by other interactions occurring at the platform, where a battery of at least ten other endocytic proteins are known to bind.

The majority of AP-2 binding partners have additional docking determinants (like modular phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P_2)-binding domains) or interaction sequences that can engage other clathrin coat components. The growing consensus is that it is the combinatorial effect of these associations that governs precise compartmental recruitment of structurally related adaptors like AP-1, AP-2 and AP-3, and epsin 1 and epsinR [68, 221, 222]. For example, epsin 1 has a PtdIns(4,5)P_2-binding amino-terminal ENTH domain, eight DPW repeats, two clathrin-binding sequences as well as NPF triplets that can bind to EH domain proteins like eps15 and intersectin [68, 147]. These additional contacts with the assembling coat

could also contribute to the steady presence of epsin 1 in clathrin-coated regions throughout the assembly and fission process. PtdIns(4,5)P_2 engagement by the epsin ENTH domain involves the ordering of a new α-helix that, once formed, inserts several aliphatic side chains into the bilayer [223]. The penetration of these residues is thought to induce membrane curvature as clathrin lattice assembly progresses [223]. We find extensive epsin 1 labeling of flat hexagonal clathrin arrays. These results indicate that the presence of epsin 1 does not obligatorily dictate membrane curvature.

A significant issue is whether, in individual cells, the entire cohort of binding partners is available to associate with the appendage or whether tissue-specific expression patterns limit the complexity of α appendage interactions. In brain, all but two of the 15 proteins shown schematically in Fig. 3.16 are expressed, and many have been directly localized to the presynaptic region, a site of intensive endocytic activity because regulated exocytosis of neurotransmitter is tightly coupled to compensatory clathrin-mediated endocytosis. Therefore, the hierarchical pattern of recruitment we see in our biochemical affinity-association assays have direct physiological relevance. A important component that is not present in brain is SJ170, the long splice-isoform of synaptojanin 1. In neurons, the critical phosphoinositide polyphosphatase function is preformed by SJ145, an alternate splice isoform that lacks the carboxy-terminal extension which contains the DPF, FXDXF and WXX[FW]X[DE] motifs [224]. A single WXX[FW]X[DE] motif is present within SJ145 but the phosphatase is expressed at considerably higher concentrations in brain than in other tissues and is predominantly recruited to the bud site by endophilin or amphiphysin, through proline-rich region–SH3 domain interactions [67]. The abundance of SJ145 in nerve terminals may therefore facilitate function without a requirement for more complex adaptor interaction information. In fact, the interaction of SJ145 with

endophilin and amphiphysin is regulated by phosphorylation [225, 226] and, because of the higher concentration of synaptojanin 1 at the synapse, the long splice isoform could conceivably be recruited prematurely to the developing bud and impede the rapid completion of the vesicle, which is dependent on PtdIns(4,5)P_2.

Many of the AP-2 binding endocytic accessory proteins are reversibly phosphorylated and regulation of the physical interaction between the α appendage and binding partners by cycles of phosphorylation/dephosphorylation could add an additional layer of control by modulating the accessibility and/or affinity of α appendage interaction motifs [227]. However, at the synapse, many of these proteins, collectively termed dephosphins [228] are coordinately dephosphorylated by calcineurin upon depolarization and Ca^{2+} influx.

In conclusion, our results identify a clear function for the α appendage sandwich subdomain in AP-2 interactions through its ability to bind the WXX[FW]X[DE] sequence. The finding that WXX[FW]X[DE] motif engagement allows other binding motifs to engage the adjacent major platform binding site suggests a potential manner to regulate α appendage occupancy. Finally, our data lead us to predict that temporal ordering of alternate adaptors and accessory proteins necessary for proper clathrin-coated vesicle assembly is governed, in part, by particular sets of α appendage interaction motifs.

3.5 ADDITIONAL DATA

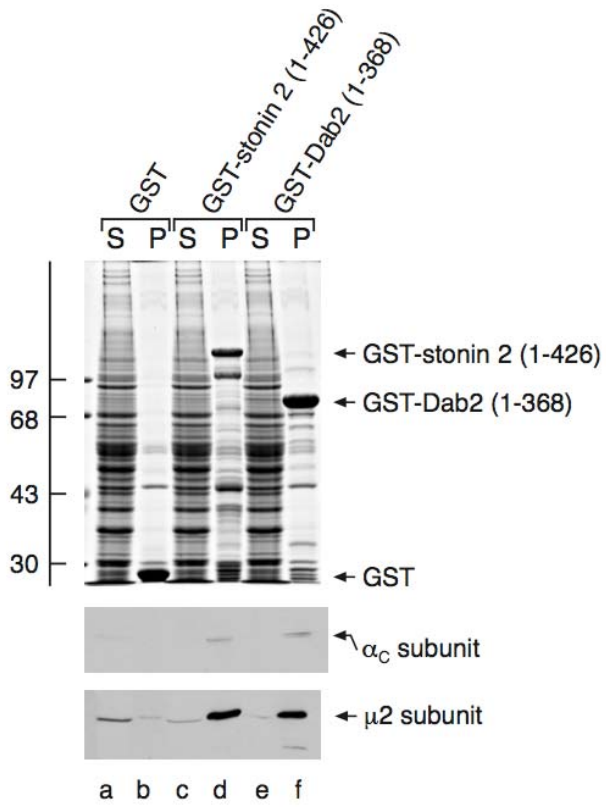


Figure 3.18: Stonin 2 interacts with AP-2.
Approximately 100 μg of either GST-stonin 2 (1-426) (lanes c and d), GST-Dab2 (1-368) (lanes e and f), or GST (lanes a and b) immobilized on GSH-Sepharose was incubated with rat brain cytosol. After centrifugation, aliquots corresponding to one-fortieth of each supernatant (S) and one-eighth of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie Blue or transferred to nitrocellulose. Portions of the blots were probed with anti-AP2 α subunit mAb 100/2 or anti-μ2 subunit serum. This data does not conclude if the interaction between stonin 2 and AP-2 is direct or indirect.

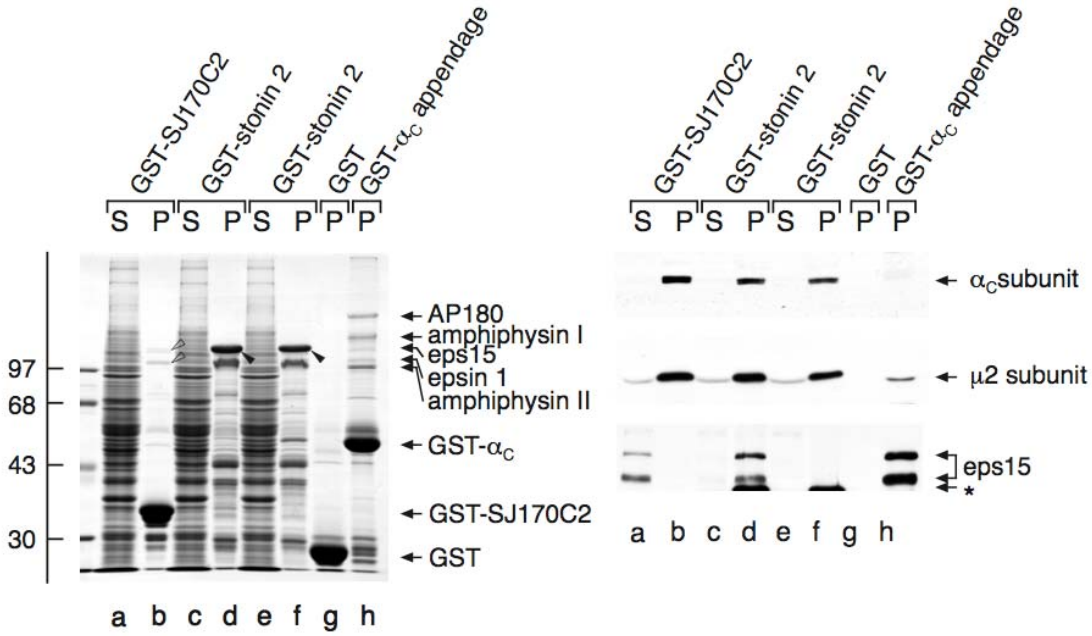


Figure 3.19: The WXXF domain of stonin 2 binds AP-2 independently of eps15.
Approximately 100 μg of either GST-SJ170C2 (lanes a and b) or GST-stonin 2 (residues 1-426) (lanes c-f) immobilized on GSH-Sepharose was incubated with either mock (GST)-depleted (lanes a-d) or GST-α_c appendage-depleted (lanes e and f) rat brain cytosol (which was prepared by preincubation with either 150 μg of immobilized GST or GST-α_c appendage; the resulting pellets (lanes g and h) demonstrate capture of AP-2 binding partners, including eps15). After centrifugation, aliquots corresponding to 1/60 of each supernatant (S) and 1/8 (lanes b, d, and f) or 1/12 (lanes g and h) of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie Blue or transferred to nitrocellulose. The position of the bound AP-2 α- and β2-subunits are indicated with open arrowheads, whereas the intact GST-stonin 2 fusion protein is indicated with filled arrowheads. Portions of the blots were probed with the anti-AP-2 α-subunit mAb 100/2, anti-AP-2 μ2 subunit, or anti-eps15 antiserum. *, cross-reactivity of the anti-eps15 antiserum with the intact GST-stonin 2 fusion protein. This data indicates that stonin 2 interacts with AP-2 directly.

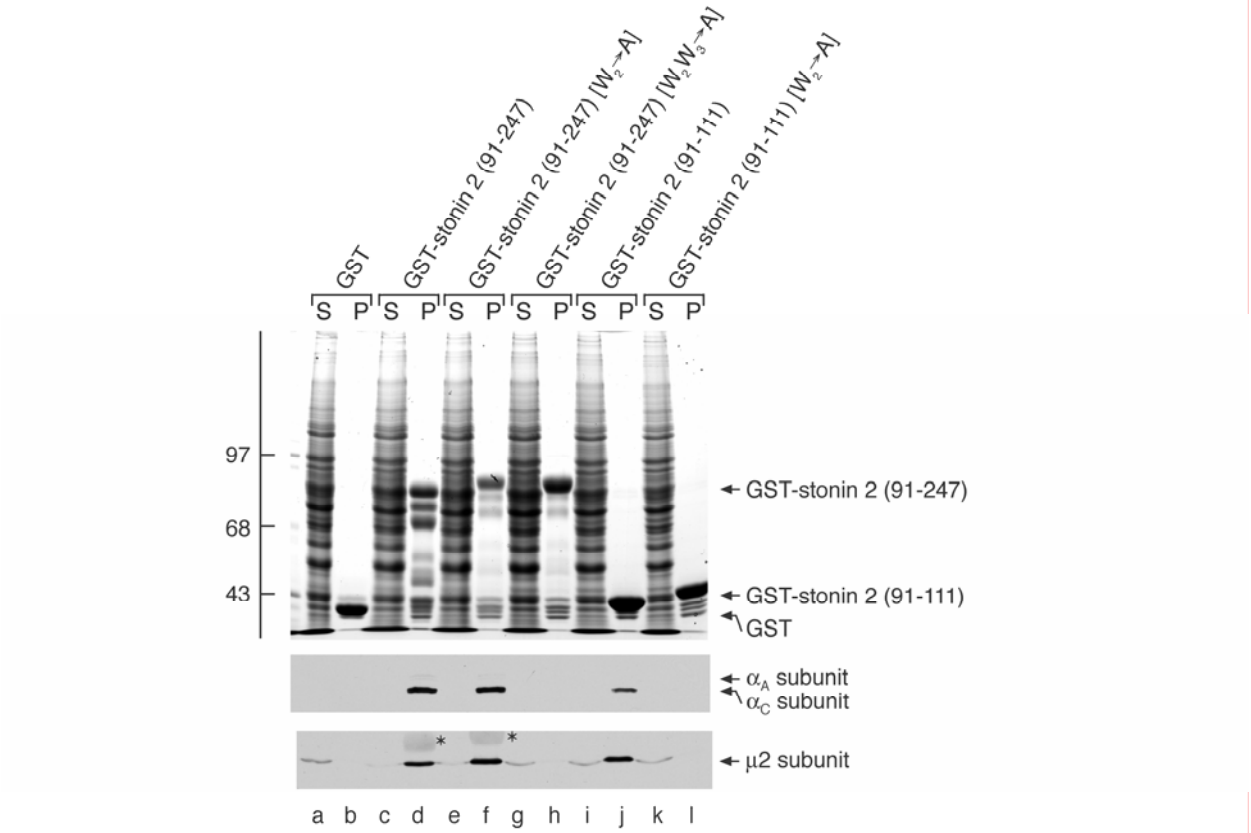


Figure 3.20: Two WXXF motifs bind AP-2 α appendage well, while one WXXF motif binds weakly.
Approximately 100 μg of either GST (lane a and b), GST-stonin 2 (residues 91-247) (lane c and d), GST-stonin 2 (91-247) [W_2->A] (lane e and f), (91-247) [W_2W_3->A] (lane g and h), (91-111) (lane i and j), or (91-111) [W_2->A] (lane k and l) immobilized on GSH-Sepharose was incubated with rat brain cytosol. After centrifugation, aliquots corresponding to 1/40 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed with the anti-AP-2 α subunit mAb 100/2 or anti-μ2 subunit serum. The asterisks indicate where protein transfer has be reduced by the large amount of GST-fusion protein comigrating at that position. This data indicates that two WXXF motifs are able to engage AP-2 well while one WXXF motif engages AP-2 weakly.

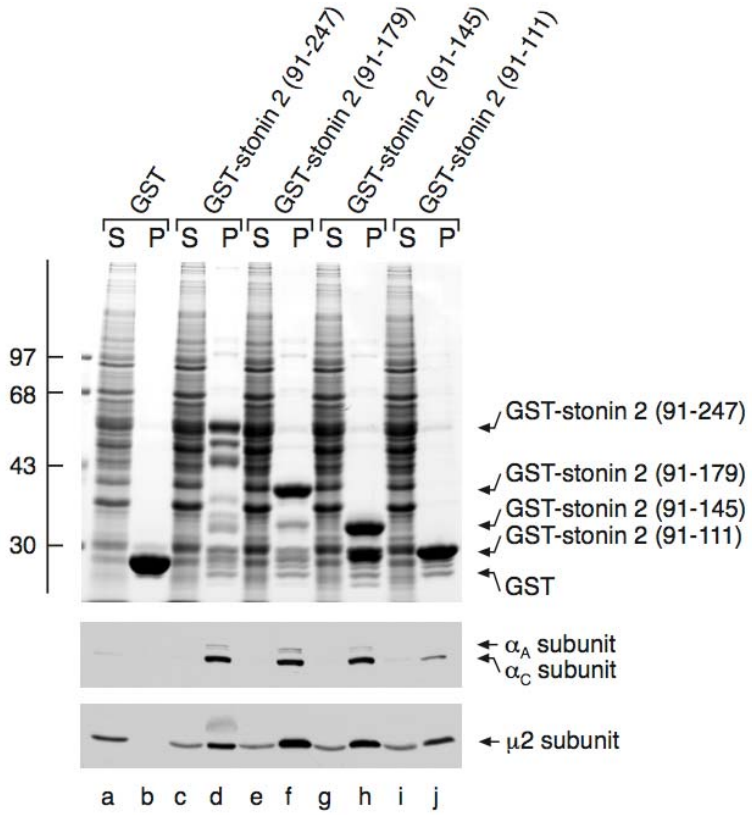


Figure 3.21: Additional binding information is contained within stonin 2 between residues 111-145.
Approximately 100 μg of either GST (lane a and b), GST-stonin 2 (residues 91-247) (lane c and d), GST-stonin 2 (91-179) (lane e and f), (91-145) (lane g and h), (91-111) (lane i and j) immobilized on GSH-Sepharose was incubated with rat brain cytosol. After centrifugation, aliquots corresponding to 1/40 of each supernatant (S) and 1/8 of each washed pellet (P) were resolved by SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose. Portions of the blots were probed with the anti-AP-2 α subunit mAb 100/2 or anti-μ2 subunit serum. This data indicates that additional binding information is contained within the stonin 2 region 111-145. Additional experiments will be required to identify the exact binding sequence.

4.0 CONCLUSIONS

4.1 INTRODUCTION

While CME is the best-studied form of endocytosis in mammalian cells, the advancements of recent years have brought our understanding well beyond the traditionally accepted model of CME. This simplified “textbook” model posits that clathrin-coated pits are assembled with the help of AP-2 at the plasma membrane surface, and cargo molecules are selected for internalization at these sites, which ultimately form vesicles that are released with the help of dynamin. Yet, over twenty accessory proteins have now been identified that bind to core CME components and participate in endocytic events, and novel sorting signals and interactions have been recognized [48, 67, 68]. Phosphorylation of AP-2 is required to enable its interaction with cargo, and the role of plasma membrane lipids in CME has also become appreciated [54, 56, 57]. These numerous advances have important implications, as understanding the molecular interactions and chronology of CME allows insights into how nutrients, extracellular macromolecules, and receptors are internalized, and how these processes are altered in pathological states.

In this dissertation, I examined several aspects of the CME process. The major theme of my work has been describing the protein-protein interactions involved in CME; specifically, the interactions of epsin 1 UIMs and stonin 2 WXXF motifs. Within these two different projects are

some unifying themes. Both epsin and stonin 2 are able to interact with the AP-2 α appendage. Epsin engages the platform site utilizing the DP[FW] repeats while stonin 2 uses its WXXF motifs at the sandwich site. Another similar theme is the endocytic motif repeats in these two proteins: three UIM repeats in epsin and three WXXF repeats in stonin 2. Functionally, these tandem repeats may enable an increased effective affinity for their binding sites, as discussed below. More globally, these protein interactions are important in the chronology of AP-2 interactions during CME. Another major theme is that both epsin 1 and stonin 2 act as CLASPs, epsin 1 for polyubiquitin cargo proteins and stonin 2 for synaptotagmin, as recently shown by the Haucke group [229].

4.2 POLYUBIQUITIN IS A BONA FIDE ENDOCYTIC SIGNAL RECOGNIZE BY THE CLASP EPSIN 1

A large portion of my research has focused on the endocytic tag ubiquitin. One question that arises is why do cells utilize multiple endocytic tags, for example YXXØ, FXNPXY, di-leucine, and ubiquitin (Figure 4.1). Possibly by embedding primary sequence motifs such as YXXØ, FXNPXY or dileucine motifs in the cytoplasmic tail of a trafficking protein, these “different endocytic classes” can be differentiated by the appropriate endocytic sorting machinery and thus allow for differential endocytosis of the cargo. Distinct endocytic pathways can be sorted from each other via their unique motifs that require various CLASPs. In addition to this explanation, having multiple signals embedded in the tail may create redundant endocytic tags and may result in an increased fidelity of endocytic uptake. Finally, ubiquitin as an internalization signal is distinct from the embedded signals in that it can be reversibly added to a target protein, in a

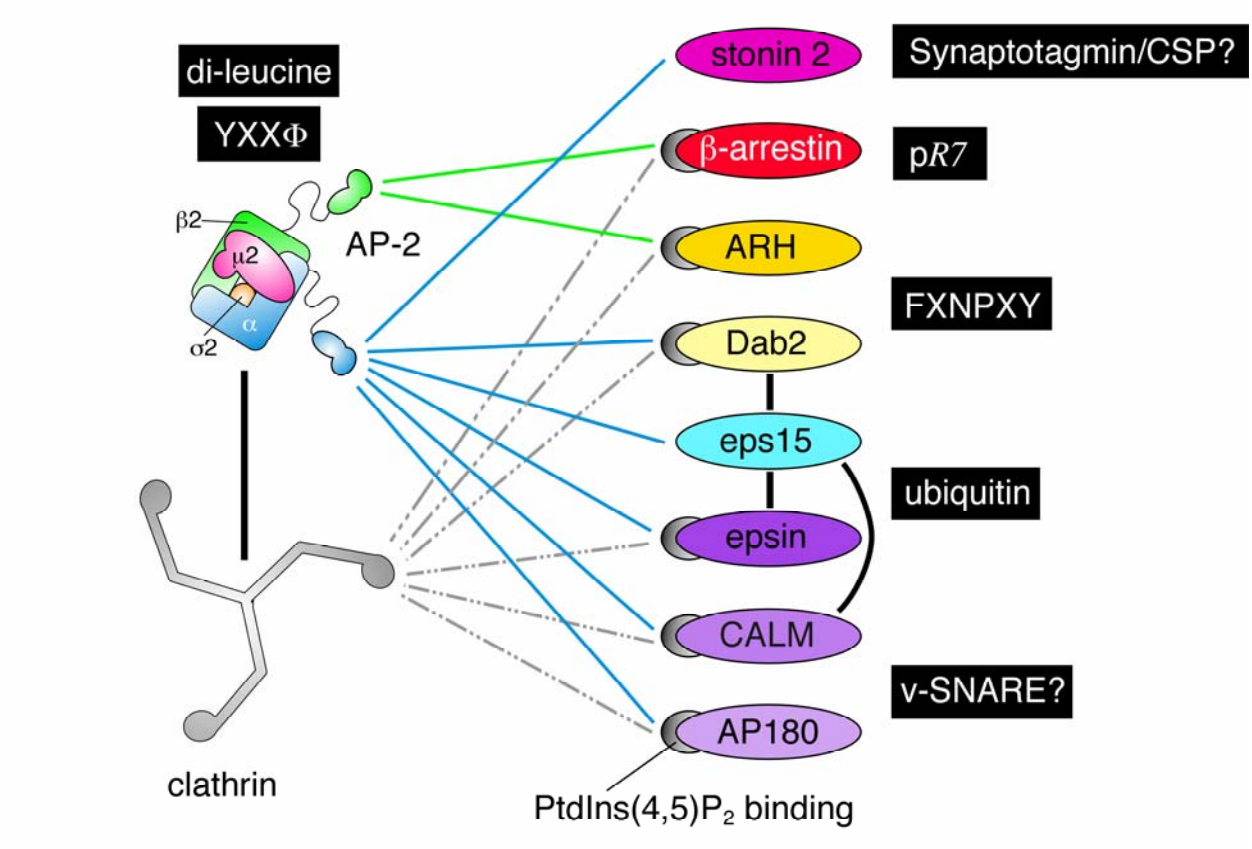


Figure 4.1: CLASP family of proteins.
Interactions between AP-2, clathrin (modeled after known structures) and other CLASPs is denoted by the lines. A PtdIns(4,5)P_2 interaction is indicated by the shaded oval. Known or probable cargo sorted by the different CLASPs is indicated in the black boxes.

similar manner as phosphorylation of GPCRs, thus imparting an endocytic signal where one may not have already existed or adding an additional signal to increase the fidelity or rate of internalization. The advantages of this type of sorting signal are discussed below.

My dissertation research has investigated and biochemically characterized the various domains in epsin 1, specifically focusing on the UIMs. I showed that these UIMs have a marked preference for polyubiquitin chains (tetraubiquitin or longer) and there is virtually no stable association with monoubiquitin. This is a particularly interesting finding because other prominent researchers in the field maintain that the ubiquitin endocytic signal in mammalian cells is a single ubiquitin, similar to yeast [145, 146].

To fortify my data in support of polyubiquitin endocytic signals, competition studies utilizing excess monoubiquitin in the presence of polyubiquitin chains were performed. These results showed that monoubiquitin is unable to compete (even at high excess) the polyubiquitin binding capacity of the UIMs. The relative affinity for polyubiquitin chains was comparable for both Lys48- and Lys63-linked polyubiquitin chains, and was qualitatively far greater than that for monoubiquitinated cargo. My results are in contradiction with data that suggests that monoubiquitination may be sufficient for internalization of receptor tyrosine kinases such as EGF receptor [125]. The Luckacs group used the monomeric type 1 membrane protein CD4 to show that only multiple ubiquitin moieties can be recognized by the clathrin endocytic machinery [166]. One possible explanation is that these receptors dimerize upon activation, and single ubiquitin moieties are attached at multiple sites on their cytoplasmic tails; perhaps this "effective multiubiquitination," though distinct from polyubiquitin chains, is enough to engage the UIMs and drive their endocytosis. In contrast, a recent publication has shown that within 2 minutes of stimulation by EGF, at least 50% of ubiquitin added to the EGF receptor is in fact

polyubiquitin, and the vast majority is the Lys63-linked form [230]. My data revealed that UIMs are able to engage both Lys48- and Lys63- linked ubiquitin chains (the lUb4 constuct used mimics Lys63-linked chains in structure). The majority of EGF receptor has Lys63 linkages, which suggests a possible mechanism for segregating the endosomal and proteasomal pathways. Traditionally, Lys48-linked ubiquitinated proteins are destined for degradation. The linkage pattern may allow for specific ubiquitin-modifying enzymes (such as ligases and deubiquitinating enzymes) to act at the surface and preferentially modulate endocytic uptake.

My contention that polyubiquitin is vital as an internalization signal for epsin-dependent CME is supported by other research groups [166, 231, 232]. In a collaborative study with the Johnson laboratory this model was tested with respect to ENaC, whose surface expression is known to be regulated by ubiquitination [231]. Using a pull-down approach, I showed that an ENaC-polyubiquitin fusion protein interacts with the epsin 1 UIM more robustly than ENaC-monoubiquitin. This finding was supported by data from the *Xenopus* oocyte model, in which epsin expression down-regulated ENaC surface activity [231].

These findings support a role for the epsin 1 and polyubiquitin-mediated CME model in physiology, which may have implications for disease. Liddle's syndrome, an inherited form of hypertension, is caused by mutations that delete a C-terminal motif in ENaC [233]. This motif is responsible for binding Nedd4-2, an E3 ubiquitin ligase that acts upon the ENaC cytoplasmic tail [233]. The resulting decrease in ENaC ubiquitination causes a reduction in ENaC CME via UIM-containing CLASPs. The functional consequence is increased ENaC at the surface of cells, which allows increased membrane sodium conductance, and ultimately the hypertension phenotype. The understanding of the ubiquitination of ENaC and its subsequent CME via UIM-

containing CLASPs could help in our understanding of disease phenotypes with similar pathophysiology.

In addition to regulation of membrane constituents such as ENaC for physiological purposes, the epsin 1 polyubiquitin internalization machinery may also be exploited by viruses in disease states. Kaposi's sarcoma, caused by the Kaposi's sarcoma herpesvirus (KSHV), is the most common cancer in AIDS patients [234, 235]. Intracellular KSHV evades recognition by the immune system by promoting the internalization of surface immunoreceptors, such as major histocompatibility class-I (MHC-I) [194, 236]. The KSHV proteins, modulator of immune recognition 1 and 2 (MIR 1/2), act as a viral E3 ligase and polyubiquitinate MHC-I to drive its endocytosis [194, 236]. It has now been shown by the Lehner group that monoubiquitinated MHC-I acts as a poor endocytic signal, while polyubiquitination acts efficiently and utilizes epsin as its adaptor protein [232]. One could extend some of the Lehner studies by researching the significance of UIM-containing proteins during the course of a KSHV infection. It will be important to show that the UIM in epsin is necessary and sufficient for MHC-I internalization in KSHV-infected cells by utilizing siRNA mediated silencing and rescue with a mutant construct. Also, other endocytic proteins may be important as evidenced by the weak effect of epsin RNAi.

A better understanding of the mechanism of MIR proteins in the pathophysiology of Kaposi's sarcoma could conceivably be used diagnostically or therapeutically. Diagnostically, assays could be performed to detect the presence and levels of the MIR proteins (or ubiquitin E3 ligase activity), which could allow for more tailored treatment regimens for Kaposi's sarcoma patients. Therapeutically, targeting of either the polyubiquitin signal or the ubiquitin sorting CLASPs such as epsin could be utilized to slow the progression of disease (e.g. by saturating endocytic machinery). There may be problems with therapeutically targeting epsin in humans,

though. Data from the De Camilli lab has shown that mice deficient in either epsin 1 or epsin 2 (both expressed in brain) do not have a gross phenotype. However, when both of these genes are disrupted simultaneously, the effect is early embryonic lethality, most likely due to defects in endocytosis during development. Similarly, other important endocytic protein deletions result in the same phenotype. Although targeting epsin for a therapeutic approach may be difficult, understanding the molecular mechanism of the ubiquitin endocytic tag and the machinery that recognizes this tag may offer insights into possible downstream targets.

Interestingly, the viral MIR proteins have homology to several endogenous mammalian proteins. Membrane-associated RING-CH (MARCH) gene products are human proteins related to MIRs and are also known to downregulate MHC-I in cells, potentially by utilizing the epsin or UIM-containing CLASP endocytic machinery [164, 237]. These proteins are very highly expressed in placental tissue, which I showed to be a site of enriched polyubituitinated cargo and epsin 1. The functions of MARCH gene products within the cell remain to be explored.

Ubiquitin as a signal has a major advantage over other endocytic motifs in that it can be easily regulated in a spatial and temporal manner. This signal can be added or removed quickly from a target protein and thus enable a signaling receptor to be rapidly cleared from the surface or remain at the surface until activated. A surface protein can exist at the plasma membrane and ligand binding or other triggers can cause its ubiquitination and subsequent internalization. For other sorting signals in which peptide motifs are embedded in the proteins, the internalization is constitutive and only controlled by steric availability or exposure of the motif.

An important system that exploits the dynamic regulation of ubiquitin-mediated CME is the Notch signaling pathway, which is highly conserved from *Drosophila* to humans and is involved in cell fate choice and pattern formation during development. Data from the *Drosophila*

model system implicates Neuralized (an E3 ligase) and Lqf (a homolog of epsin containing UIMs) in the modulation of Notch signaling through Delta (the transmembrane ligand of Notch) endocytosis [238]. Endocytosis was first recognized as a regulatory mechanism when the dynamin mutant *shi*^{ts} was shown to be necessary in both the signal generating cell (Delta expressing cell) and the signal receiving cell (Notch receptor expressing cell) for normal Notch mediated signaling [239]. The dynamic nature of ubiquitin addition and removal makes it an ideal internalization signal during development. If Delta had a primary amino acid sequence endocytic signal, this would cause its constitutive endocytosis. Conversely, the ability of the ubiquitin signal to be dynamically added to Delta enables its internalization at specific stages by CME. Another key aspect of the *Drosophila* model is that in sensory organ precursor (SOP) cells, numb, an endocytic CLASP that is thought to engage a binding partner of Notch known as Sanpodo (Spdo), is able to sort one type of cargo while Lqf is able to sort another (ubiquitinated cargo). This is an important example because in the same cell, different CLASPs are able to sort different cargo molecules simultaneously.

Another intriguing ubiquitinated protein system involves colony stimulating factor 1 (CSF-1), the primary regulator of macrophage and osteoclast development which is mediated through the CSF-1 receptor [153, 240]. CSF-1 receptor is a receptor tyrosine kinase and when activated recruits Cbl, which then ubiquitinates CSF-1 receptor to induce the receptor's internalization [153, 240]. This activity can attenuate macrophage proliferation and is vital for proper immune system function, which also requires dynamic modulation in a manner that only ubiquitin as a sorting signal provides.

In addition to studying the role of UIM-containing CLASPs in the regulation of physiologic protein internalization, much work remains in the study of the CLASPs themselves.

It is intriguing that while able to bind ubiquitin, epsin is also a substrate for covalent monoubiquitination itself [241, 242]. I think it would be interesting to explore the effects of the state of ubiquitination of epsin on its interactions with ubiquitinated cargo or with coat components. One possibility is that the ubiquitination state may influence its ability to bind to polyubiquitinated cargo [120].

To study the role of the ubiquitination of epsin, one could first map and then mutate its ubiquitination sites, express the constructs in cells with silenced endogenous epsin, and look for changes in internalization of various substrates. However, these experiments may become quite complicated because there are multiple UIM-containing CLASPs that may have redundant effects, such as epsin 2, eps15, and eps15R (which are also known to be ubiquitinated themselves). Among the challenges to appropriately explore this question are that it may require silencing of many endogenous endocytic CLASPs as well as other accessory proteins to determine the true implications of a mutation in one of these proteins. This is a likely necessity, as the epsin 1 silencing experiment performed by Lehner's group revealed only a mild phenotype [232].

An important regulator of ubiquitinated processes is deubiquitination, and it might be expected that deubiquitinating enzymes are present and active at the clathrin-coated pit to regulate ubiquitin-mediated CME. Upon the deletion of the main deubiquitinating enzyme in *S. cerevisiae* (Doa4), many plasma membrane proteins are stabilized [243]. It is thought that this phenotype is a result of depletion of cellular free ubiquitin, causing impaired ubiquitination [243]. The importance of ubiquitination as a signal for CME begs the question whether there is a deubiquitinating enzyme that is active at clathrin coated pits. Such an enzyme might modulate internalization of ubiquitinated cargo and provide an additional layer of regulation for CME of

important surface cargo. One recently identified candidate is associated molecule with the SH3 domain of STAM (AMSH) protein. AMSH has been shown to have a role in the deubiquitination of endosomal cargo preceding lysosomal degradation [244]. Another possible candidate protein that has deubiquitination activity is ataxin-3. In addition to this activity, ataxin-3 has two UIMs, which are known to bind to polyubiquitin and not mono- or diubiquitin moieties [177, 245]. Another approach is to perform an extensive database search in a manner similar to that which identified epsin as a UIM-containing protein. Using a computer algorithm that I developed, I have performed a preliminary database search of proteins that contain DP[FW], WXXF, FXDXF, NPF, clathrin binding, and β appendage binding motifs, which are known endocytic interaction motifs, to broadly search for other proteins that are localized to clathrin-coated pits. Interestingly, I noticed that among known binding proteins, this search returned candidates that included several proposed ubiquitin proteases (See Appendix A). In order to confirm that these are functioning at clathrin coated pits, it would be important to perform biochemical experiments to verify interactions with clathrin, AP-2, and lipids. Other important studies are to assay their catalytic activity and localize the putative deubiquitinating enzymes to clathrin-coated pits.

The study of deubiquitinating enzymes may help us understand how the same signal (tetraubiquitin or longer chains) allows for endocytosis at the surface and degradation in the cytosol. Deubiquitinating enzymes are potentially able to act rapidly at the surface once the ubiquitinated cargo protein is sorted into a clathrin-coated pit. The computer search results (shown in Appendix A) identify many possible deubiquitinating enzymes containing multiple endocytic motifs allowing for these enzymes to localize preferentially to the surface and act rapidly to deubiquitinate the cargo once sorted into a pit. In this manner, polyubiquitinated

endocytosed cargo may be deubiquitinated upon internalization prior to its recognition by the degradation machinery.

4.3 PROTEIN-PROTEIN INTERACTIONS DICTATE THE CHRONOLOGY OF AP-2'S INVOLVEMENT DURING CME

Recent progress has clearly improved our understanding of the precise molecular chronology that occurs during the creation, progression, scission, release, and uncoating of a clathrin-coated vesicle. This rapid assembly and disassembly is governed by protein-protein interactions that are carried out in a strict spatial and temporal sequence to ensure vectoral progress. My work has detailed the role of epsin as a CLASP and has enhanced our understanding of the biochemical interactions between ubiquitinated cargo and the endocytic machinery. Another of my research endeavors focused on AP-2 and its interaction with stonin 2, a protein that had been examined in the *Drosophila melanogaster* model system but was relatively unstudied in mammalian cells.

My work has described a novel α appendage binding sequence and cognate contact surface, known as the β-sandwich site. A common question throughout this work is why do proteins have several sequences and multiple sites for α appendage engagement? Having distinct sites with different affinities can account for the fidelity of incorporating endocytic components at the bud site. In addition to increasing fidelity, these sites can enable a chronology of events, with early-acting proteins being displaced by later-acting proteins that possess multiple higher-affinity engagements for the appendage binding sites. Another advantage to having multiple sites

is that this can create a web of interactions at the bud site and enable an increased local concentration of endocytic components based on these interactions.

The AP-2 α appendage WXXF binding sequence occurs in three tandem repeats in stonin 2. I became interested in this protein due to its arrangement of arrayed endocytic motifs (similar to my other project involving epsin 1, which has tandem UIMs, along with tandem AP-2 interacting DP[FW] motifs), and the potential of stonin 2 as a sorting adaptor. Through studies described in chapter 3, it was shown that there are two surfaces on the AP-2 α appendage and at least three distinct binding sequences that allow for interactions with proteins that provide diverse functions including sorting, lattice assembly, structural, and enzymatic functions.

Proteins with multiple, tandemly arrayed AP-2 interaction motifs (such as epsin 1, which has eight DPW sequences) can engage several AP-2 α and β2 appendage subunits simultaneously. This strategy, of utilizing weak binding of tandemly arrayed motifs, ensures that the protein has a high apparent affinity: when the protein diffuses, the multiple motifs enable an increased probability of rebinding the same site. The affinity of the WXXF motif for the β sandwich-binding site (~10 μM) is an order of magnitude greater than the affinity of the platform binding sequences (~120 μM) for their respective binding sites. In this manner, tandemly arrayed low-affinity interaction sequences allow for a "kinetic trap:" once a protein binds to its substrate, multiple interactions retain the interaction of the two proteins despite cycles of binding and release. Also, the low affinity reactions allow for easy displacement by a higher affinity interaction and thus a coordination of events. If the proteins had one motif with a much higher affinity it would be difficult to disassemble many of these interaction webs that the endocytic process is known to create.

This difference in “platform” site binding affinity, as well as the competition of many proteins with “platform” binding sequences versus few proteins possessing β-sandwich-binding sequences, allows for the β-sandwich site to behave as a “privileged” surface on the AP-2 α appendage. Proteins possessing these privileged sequences can be incorporated into developing buds with a higher fidelity compared to proteins competing for one of the “platform” sites.

A physiologically relevant example of this “privileged” site system occurs at the synapse. Stonin 2 is a brain-enriched endocytic protein that in *D. melanogaster* (stonedB) has been linked to the function of synaptotagmin 1, an important mediator of calcium dependent neurosecretion at the synaptic plasma membrane [246]. My studies biochemically characterized the endocytic motifs embedded within stonin 2 and demonstrated its capacity to function as a potential sorting adaptor. Stonin 2 is an atypical member of the CLASP family though, as it does not possess a clathrin binding or membrane engagement domain. It instead has the tandemly arrayed WXXF motifs that enable its interaction with AP-2, and a μ homology domain (MHD), which enables its interaction with its putative cargo, the synaptic vesicle protein synaptotagmin 1. Recently, the Haucke group extended my findings on stonin 2, demonstrating that this protein is indeed able to specifically sort synaptotagmin 1 into clathrin-coated pits [229].

Another putative class of cargo proteins that may be sorted by stonin 2 are the cysteine string proteins (CSPs). CSPs contain a ”J” domain and an unusual cysteine rich region. In *Drosophila,* CSPs were shown to associate with synaptic vesicles and were mislocalized, along with synaptotagmin, in stonedB mutant flies [247]. It would be interesting to study the potential of stonin 2 to act as a dedicated sorting adaptor for CSP in addition to synaptotagmin. To further confirm stonin 2’s role as a sorting adaptor for synaptotagmin 1 and CSP, one could utilize siRNA to silence stonin 2 in a relevant cell type (such as a neurons) and observe the cargo

proteins. Synaptotagmin is an important protein to retrieve from the surface because it is a synaptic vesicle (SV) protein involved in SV fusion with the plasma membrane and is critical in exocytosis and neurotransmitter release.

While many proteins are known to engage AP-2, it is possible that additional AP-2 interacting proteins with interesting functions in CME have yet to be described. To identify these proteins, one could utilize the technique of phage display, in which peptides are fused to capsid proteins on the phage surface. Libraries of these phage-displayed peptides can then be screened for binding and the exact peptide sequence can be determined. This approach would further characterize interactions of the WXXF motif within AP-2 to recognize peptides with affinities that may be relevant for CME. Additionally it could be used to identify anchor residues, or possibly even novel sequences that do not occur in the proteome for use to modify CME. Alternatively, phage or peptide display could be used to identify novel binding sites and substrates for both the AP-2 α and β2 appendages, which are where most endocytic protein-protein interactions occur.

The dual site on AP-2 is based on affinity, in which a higher-affinity binding partner displaces a lower-affinity interaction. There may be additional mechanisms that regulate the hierarchy of binding to AP-2 which act on top of the dual site system. One mechanism for regulation of binding that is utilized by endocytic proteins is post-translational modification, such as phosphorylation. In fact, it is known that several endocytic proteins are phospho-proteins, and their phosphorylation may regulate their ability to interact with AP-2. These phosphorylation reactions, among other post-translational modifications, may add another level of complexity and regulation to the system.

4.4 CLOSING COMMENTS

In this dissertation, I presented data that has furthered our understanding of the biochemical nature and chronology of the protein-protein interactions that are important during CME. The recognition of epsin as a bona fide CLASP and subsequent identification of poly-ubiquitination as an endocytic signal have important implications for pathological states that involve this machinery to impact the cell. The identification of AP-2 interaction sites has allowed a greater understanding of the sequence and mechanism of cargo engagement during CME. My research has raised questions regarding the importance of multiple sorting signals, multiple protein interaction motifs within endocytic proteins, and regulation of these interactions. CME is critical in many physiological processes, and future studies may help us better understand its function in normal physiology, its disruption in pathophysiology, and its potential for manipulation in therapeutic approaches.

5.0 MATERIALS AND METHODS

5.1 CHAPTER 2 MATERIALS AND METHODS

5.1.1 Recombinant DNA Manipulations

All epsin constructs were prepared from a rat epsin 1 cDNA kindly provided by Pietro de Camilli (Yale University School of Medicine, New Haven, CT, USA). Generation of GST-epsin 1 (1-407) has been described [248] while the plasmid encoding His6-epsin 1 (144-575) was kindly provided by Ernst Ungewickell (Hannover Medical School, Hannover, Germany). GST-eps15 UIM (residues 828-896) was prepared by PCR using a human eps15 cDNA kindly provided by Alexander Sorkin (University of Colorado Health Science Center, Denver, CO, USA) as the template, and ligated into pGEX-4T-1. GST-ubiquitin was prepared similarly by PCR using a monoubiquitin cDNA as a template, while GST-linear tetraubiquitin was generated by inserting a single in-frame ubiquitin between the *Bam*HI and *Not*I sites at the end of a GST-triubiquitin plasmid kindly provided by Gergely Lukacs (Hospital for Sick Children, Toronto, Canada). The first three of these tandemly-arrayed ubiquitins have Gly76 substituted for Val to prevent cotranslational cleavage by ubiquitin-specific processing proteases. The GST-S5a plasmid was a gift from Martin Rechsteiner (University of Utah, Salt Lake City, UT, USA). Tac (CD25; the IL-2 receptor a subunit) in pcDNA3 was a gift from Rebecca Hughey (University of Pittsburgh, Pittsburgh, PA, USA). The Tac_L-Ub in pcDNA3 was constructed by first extending the 11-amino

acid cytosolic domain of Tac to the linker sequence (Tac_L) RRQRKGGGSGGGTGGGSGKSRRTLEAAAAAI incorporating terminal, adjacent *Xho*I and *Not*I restriction sites. A single ubiquitin, an all-arginine-substituted (K7R) ubiquitin kindly provided by Cecile Pickart (Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA), or the linear, ubiquitin protease-resistant tetraubiquitin was then inserted into the modified Tac_L plasmid using the *Xho*I and *Not*I sites. To prevent activation by the E1 ubiquitin-activating enzyme, the terminal Gly pair of the final ubiquitin in these vectors were removed using site-directed mutagenesis to convert Gly75 to a stop codon. All constructs were verified by automated dideoxynucleotide sequencing and full oligonucleotide and sequence details are available upon request.

5.1.2 Protein Preparations and Purification

Lys48- or Lys63-linked polyubiquitin chains were purchased from Biomol (Plymouth Meeting, PA, USA) or Boston Biochem (Boston, MA, USA). The preparation of clathrin-coated vesicles, clathrin, AP-2, and GST-fusion proteins has been described [248]. Briefly, coated vesicles were purified in buffer A (100 mM MES-OH, pH 6.5, 1 mM EGTA, 0.5 mM magnesium chloride, and 0.02% sodium azide) from homogenized tissue using sequential differential centrifugation followed by a 12.5 % Ficoll 400/12.5% sucrose isopycnic step. The placental preparation was further purified by chromatography over a Sephacryl S-1000 column equilibrated in buffer A to remove smooth membrane contaminants. Coat proteins were extracted from coated vesicles with ~1 M Tris-HCl, pH 7.0 and fractionated on a Superose 6 column equilibrated in 0.5 M Tris-HCl, pH 7.2, 0.1% β-mercaptoethanol, and 0.02% sodium azide. The clathrin peak was pooled while the adaptor peak was further fractionated on a hydroxylapatite column using a linear gradient of

2-500 mM potassium phosphate, pH 8.0 in 20 mM Tris-HCl, 10% glycerol to prepare purified AP-2. Prior to use, clathrin and AP-2 were gel-filtered into assay buffer (25 mM HEPES, pH 7.2, 125 mM potassium acetate, 5 mM magnesium acetate, 2 mM EDTA, 2 mM EGTA, 1 mM DTT). GST and the various GST fusion proteins were all expressed in *E. coli* strain BL21, while the His6-epsin 1 (144-575) was produced in the Rosetta strain (Novagen, San Diego, CA, USA). After lysis, GST fusions were purified using glutathione-Sepharose by standard procedures and dialyzed into PBS. The preparation of HeLa cell lysates and rat brain Tris-Triton lysates has been described elsewhere [248]. To examine the expression of the Tac chimeras in transiently transfected HeLa cells, PBS-washed cells were lysed in 25 mM HEPES-OH, pH 7.2, 250 mM sucrose, 2 mM EDTA, 2 mM EGTA, 0.5% Triton X-100, 10 mM *N*-ethylmaleimide, 20 mM MG132, 1 mM PMSF, and Complete protease inhibitor cocktail on ice for 30 min. Lysates was scraped off the culture dishes and then centrifuged at 10,000 × g_{max} for 10 min at 4°C. Supernatants were collected and used for immunoblotting.

5.1.3 Protein Binding Studies

Pull down-type assays, typically in 300 ml total volume, contained 5-100 μg of GST or the appropriate GST-fusion protein first immobilized upon ~25 ml packed GSH-Sepharose by incubation at 4°C. The Sepharose beads containing the required immobilized proteins were washed and resuspended to 50 ml in assay buffer. Clarified rat brain cytosol or Tris-Triton extract, HeLa cell lysate, or ubiquitin chains (in the presence of 0.1 mg/ml carrier BSA) were added and the tubes incubated at 4°C for 60 min with continuous gentle mixing. The GSH-Sepharose beads were then recovered by centrifugation at 10,000 × g_{max} at 4°C for 1 min and an aliquot of each supernatant removed and adjusted to 100 ml with SDS-sample buffer. After

washing the GSH-Sepharose pellets 4 times each with ~1.5 ml ice-cold PBS by centrifugation, the supernatants were aspirated and each pellet resuspended in SDS-sample buffer. Liposome binding assays used 0.4 mg/ml synthetic multilamellar liposomes composed of 35% phosphatidylcholine, 35% phosphatidylethanolamine, 10% phosphatidylserine, 10% cholesterol and 10% PtdIns(4,5)P_2, which were recovered by centrifugation at 20,000 × g_{max} for 20 min at 4°C. For cage binding experiments, preassembled clathrin cages, prepared by dialysis of Superose 6-column purified clathrin into buffer A supplemented with 3 mM calcium chloride, were used.

5.1.4 Antibodies and Immunoblotting

Affinity-purified polyclonal antibodies directed against epsin 1, Dab2, the cation-independent mannose 6-phosphate receptor (CI-MPR), and the anti-AP-1/2 β1/β2 subunit and anti-AP-1 μ1 subunit antibodies designated GD/2 and RY/1, respectively, were produced in our laboratory by standard procedures. Affinity-purified anti-eps15 antibodies and serum were generously provided by Ernst Ungewickell, polyclonal anti-AP-2 m2 subunit and anti-Tac antisera were generously provided by Juan Bonifacino (NIH, Bethesda, MD, USA), the anti-Hrs serum was a kind gift from Harald Stenmark (Norwegian Radium Hospital, Oslo, Norway), the anti-EEA1 antibodies were kindly provided by Silvia Corvera (University of Massachusetts Medical School, Worchester, MA, USA), and anti-LRP1 antibodies were a gift from Guojun Bu (Washington University School of Medicine, St. Louis, MO, USA). Polyclonal anti-ubiquitin-protein conjugate antibodies were purchased from Biomol, affinity-purified polyclonal anti-caveolin-1 (N-20) from Santa Cruz Laboratories (Santa Cruz, CA, USA), anti-S5a antiserum from Boston Biochem, and polyclonal anti-EGF receptor antibody from Cellular Signaling Technology

(Beverly, MA, USA). The anti-AP-2 α subunit mAb AP.6 hybridoma was kindly provided by Frances Brodsky (UCSF, San Francisco, CA, USA), the neuronal-specific anti-clathrin light chain mAb Cl57.3 was provided by Reinhard Jahn (Max-Planck-Institute for Biophysical Chemistry, Göttingen, Germany), anti-actin mAb C4 by Dorothy Schafer (University of Virginia, Charlottesville VA, USA), and the anti-insulin receptor b subunit mAb CT-1 by Guillermo Romero (University of Pittsburgh, PA, USA). Anti-ubiquitin mAb Ubi-1 was purchased from Novus Biologicals (Littleton, CO, USA), the anti-ubiquitin mAb FK2 from Biomol, anti-caveolin-1 mAb 2297 and anti-AP180 mAb 34 from BD Transduction Laboratories (Lexington, KY, USA), the anti-E1 mAb from Covance (Berkeley, CA, USA), and the anti-Tac mAb 7G7B6 from Ancell Immunology Research Products (Bayport, MN). Alexa dye-conjugated secondary antibodies were from Molecular Probes (Carlsbad, CA, USA) and Cy5-coupled antibodies from Jackson ImmunoResearch (West Grove, PA, USA).

Samples were resolved on polyacrylamide gels prepared with an altered acrylamide:bis-acrylamide (30:0.4) ratio stock solution. After SDS-PAGE, proteins were either stained with Coomassie blue or transferred to nitrocellulose in ice-cold 15.6 mM Tris, 120 mM glycine. Blots were usually blocked overnight in 5% skim milk in 10 mM Tris-HCl, pH 7.8, 150 mM NaCl, 0.1% Tween 20 and then portions incubated with primary antibodies as indicated in the individual figure legends. After incubation with HRP-conjugated anti-mouse or anti-rabbit IgG, immunoreactive bands were visualized with enhanced chemiluminescence.

5.1.5 Cell Culture, Transfection, Immunofluorescence, and Electron Microscopy

HeLa SS6 cells (kindly provided by Ernst Ungewickell), A431 cells (ATCC CRL-1555) and NRK cells (ATCC CRL-1570) were grown in DMEM supplemented with 10% fetal calf serum

and 4 mM L-glutamine in a humidified atmosphere containing 5% CO_2. Typically, cells were plated onto 12-mm round glass cover slips prior to stimulation with EGF or transfection. Alexa488-conjugated EGF and Alexa568-conjugated transferrin were from Molecular Probes. Transfections with Lipofectamine 2000, using 500 ng plasmid DNA/35-mm dish, was as recommended by the manufacturer. Approximately 14-20 hours post-transfection, cells were fixed either with 2-4% paraformaldehyde in PBS for 10-20 min at room temperature or with methanol at -20°C for 5 min. Alternatively, prior to fixation, the cells were incubated in DMEM, 0.5% BSA, 25 mM HEPES, pH 7.2 at 37°C for 60 min for transferrin uptake, or overnight for EGF uptake experiments. EGF (100 ng/ml), transferrin (25 μg/ml) and/or anti-Tac mAb (4 μg/ml) were then incubated with the cells on ice for 60 min before warming to 37°C for the indicated period prior to fixation. In some experiments, antibodies were added directly to the cell culture medium at 37°C and, after 5 or 10 min, the cells cooled rapidly on ice-water and washed in ice-cold PBS. The cells were then fixed in 2% paraformaldehyde in PBS, blocked with 10% normal goat serum in PBS, and then incubated with Alexa568-conjugated anti-mouse IgG. After washing, the cells were refixed and permeabilized with 0.2% saponin and then incubated with Alexa488-conjugated anti-mouse IgG.

All confocal images were acquired using an Olympus Fluoview 500 microscope equipped with a 60X 1.40 NA objective and a 560 nm short pass filter with a 505 nm barrier filter for the 488-nm argon laser line, a 630 short pass filter and 560-600 nm barrier filter, and a 660-nm long pass filter for the 543- and 633-nm helium-neon laser lines, respectively. Signals in the different emission channels were collected sequentially and processed either with Photoshop (Adobe Systems Incorporated, San Jose, CA, USA) or Imaris (Bitplane AG, Saint Paul, MN, USA) for 3-dimensional reconstructions from acquired X-Z image stacks.

For freeze-etch immunogold analysis, cells were cultured on small oriented pieces of carbon-coated glass cover slip and ruptured by brief sonication to generate 'unroofed' cell cortices. After washing in 30 mM HEPES-OH, pH 7.3, 70 mM potassium chloride, 5 mM magnesium chloride and 3 mM EGTA (KHMgE buffer), the cells were fixed in 2% paraformaldehyde, 0.025% glutaraldehyde in KHMgE, quenched with 50 mM ammonium chloride, 50 mM L-lysine in KHMgE, and blocked with 1% BSA in KHMgE. Coverslips were then incubated with affinity-purified anti-epsin antibodies followed by anti-rabbit antibodies conjugated to 15 nm colloidal gold. After washing in KHMgE, the membranes were refixed in 2% glutaraldehyde in KHMgE and prepared for freeze-etch analysis [119, 249].

5.2 CHAPTER 3 MATERIALS AND METHODS

5.2.1 DNA and Plasmids

The GST-α_C appendage and GST-α_C appendage mutants W840A, R905A, R916A, GST-SJ170M1, GST-SJ170C2, GST-SJ170C2 mutants (FD$\rightarrow$A) and (W$\rightarrow$A), and GST-stonin 2 (1-426) have been described previously [69, 72, 82]. The GST-α_C appendage mutants R707S, N712Y, G725N, K727A, R731A, F740D, and Q782A, and the GST-stonin 2 (1-247), -(1-117), -(1-30), -(1-247) [$W_3\rightarrow$A], -[$W_2\rightarrow$A], -[$W_1\rightarrow$A], -[$W_{2,3}\rightarrow$A], and -[$W_{1,2,3}\rightarrow$A] constructs were generated by site-directed mutagenesis using the QuikChange system (Stratagene) and the appropriate mutagenic primers (the sequences of which are available upon request). The full-length GFP-stonin 2 construct was generated by inserting the missing amino terminal residues into a GFP-stonin 2 vector [214] kindly provided by Juan Bonifacino. First, the internal *Bcl*I site

in the GFP-stonin 2 (204-905) plasmid was inactivated by QuikChange mutagenesis. Then, residues 1-426 of stonin 2 were amplified by PCR from human clone C14981 (Stratagene) with primers 5'-TTAAGCTTATATGACTTTGGACCATGTG-3' and 5'-AAGCGGCCGCCTAGTCACGAGGCTGGGACCG-3', digested with *Hin*dIII and *Bcl*I, purified, and ligated into the *Hin*dIII and *Bcl*I digested GFP-stonin 2 plasmid. This procedure inserts residues 1-393, generating full-length stonin 2 (1-905) fused in frame to the carboxyl terminus of GFP in pEGFP-C1. The GFP-stonin 2 [$W_{2,3}\rightarrow A$] was generated as outlined above. All the clones and mutations were verified by automated dideoxynucleotide sequencing.

5.2.2 Recombinant Proteins, Cell Extracts and Antibodies

GST and the various GST-fusion proteins were produced in *E. coli* BL21. The standard induction protocol entails shifting log-phase cultures (A_{600} ~0.6) from 37°C to room temperature and then adding isopropyl-1-thio-b-D-galactopyranoside to a final concentration of 100 mM. After 3-5 hours at room temperature with constant shaking, the bacteria were recovered by centrifugation at 15,000 × g_{max} at 4°C for 15 min and stored at -80°C until used. Bacteria were lysed on ice in 50 mM Tris-HCl, pH 7.5, 300 mM sodium chloride, 0.2% (w/v) Triton X-100, 10 mM b-mercaptoethanol with sonication or in B-PER reagent (Pierce). Insoluble material was removed by centrifugation at 23,700 × g_{max} at 4°C for 15 min and then the GST fusions collected on glutathione (GSH)-Sepharose. After extensive washing in PBS, GST fusions were eluted with 10 mM Tris-HCl, pH 8.0, 10 mM GSH, 5 mM DTT on ice and dialyzed into PBS, 1 mM DTT before use in binding assays. For some experiments, proteins were cleaved off the GST with thrombin and then PPACK (D-Phe-Pro-Arg chloromethyl ketone, Calbiochem), an irreversible

thrombin inhibitor, added to 25 mM. For quantitative affinity measurements, thrombin-cleaved α_C appendage was further purified by gel filtration over a Superdex S200 column.

Cytosol was prepared from frozen rat brain (PelFreez) by sequential differential centrifugation after homogenization in 25 mM Hepes-KOH, pH 7.2, 250 mM sucrose, 2 mM EDTA and 2 mM EGTA supplemented with 1 mM PMSF and Complete (Roche) protease inhibitor cocktail. The 105,000 × g_{max} supernatant is defined as cytosol and was stored in small aliquots at –80°C. PC12 cell lysates were prepared after collecting the cells by trypsinization and washing with PBS. Pelleted PC12 cells were solubilized in 25 mM Hepes-KOH, pH 7.2, 250 mM sucrose, 2 mM EDTA and 2 mM EGTA supplemented with 1% Triton X-100 on ice for 30 min in the presence of Complete (Roche) protease inhibitor cocktail and 1 mM PMSF. Following centrifugation at 20,000 × g_{max} at 4°C for 15 min, aliquots of the lysate were stored frozen at –80°C. Before use, thawed samples of either rat brain cytosol or PC12 cell lysates were adjusted to 25 mM Hepes-KOH, pH 7.2, 125 mM potassium acetate, 5 mM magnesium acetate, 2 mM EDTA, 2 mM EGTA and 1 mM DTT (assay buffer) by addition of a 10X stock and then centrifuged at 245,000 × g_{max} (TLA-100.4 rotor) at 4°C for 20 min to remove insoluble particulate material.

Polyclonal serum against NECAP 1 was generously provided by Peter McPherson, while the anti-m2 subunit serum was a generous gift from Juan Bonifacino. Polyclonal antibodies against sorting nexin 9 (SNX9) were generated in rabbits using residues 1-240 of SNX9 as the antigen. Affinity purified anti-numb antibodies were generously provided by Kozo Kaibuchi. Affinity purified rabbit antibodies to epsin 1, Disabled-2 (Dab2), and synaptojanin 1 (recognizing both SJ145 and SJ170) have been described [72, 75, 82]. The anti-AP-2 a subunit monoclonal 100/2 was a generous gift of Ernst Ungewickell and the anti-CALM mAb a generous

gift from Jeong-Ah Kim, while monoclonal antibodies directed against AP180 and amphiphysin were from BD-Transduction Laboratories. The anti-HIP1 mAb 1C5 was from Novus Biologicals.

5.2.3 Protein Binding Studies

Pull-down-type assays, in 300 ml total volume, were performed as described [72, 82]. Typically, 50-400 μg of GST and the GST-fusion proteins were first each immobilized upon ~25 ml packed GSH-Sepharose by incubation at 4°C for 2 hours with continuous mixing. The Sepharose beads containing the required immobilized proteins were then washed and resuspended to 50 ml in assay buffer. Clarified rat brain cytosol, PC12 cell lysates, or purified, thrombin-cleaved α_C appendage (in the presence of 0.1 mg/ml carrier BSA) were added and the tubes incubated at 4°C for 60 min with continuous gentle mixing. For the competition assays, a WXX[FW]X[DE] peptide (ISNWVQFEDDTP) or thrombin-cleaved proteins (in the presence of 25 mM PPACK) were added directly into the assay mixture. The GSH-Sepharose beads were then recovered by centrifugation at 10,000 × g_{max} at 4°C for 1 min and an aliquot of each supernatant removed and adjusted to 100 ml with SDS-sample buffer. After washing the GSH-Sepharose pellets 4 times each with ~1.5 ml ice-cold PBS by centrifugation, the supernatants were aspirated and each pellet resuspended in SDS-sample buffer.

5.2.4 Isothermal Titration Calorimetry (ITC) Experiments

The SJ170 KGWVTFEE peptide was synthesized in the laboratory of Paul M. Allen (Washington University). Superdex S200-purified α_C appendages were concentrated and then

proteins and peptides prepared for ITC by overnight dialysis against a buffer of 50 mM phosphate, pH 7.5, 100 mM sodium chloride, and 1 mM TCEP. All ITC experiments were carried out at 30º C using a VP-ITC instrument (MicroCal) at Washington University. Typically the cell contained 1.4 ml of 100 mM a appendage while SJ170 WXXF peptide, at a concentration of 1 mM, was titrated in 30 injections of 10 ml each. The exception was the a-appendage R905A mutant, which, due to low expression and solubility, was run with a protein concentration of 30 mM and peptide concentration of 0.90 mM. Traces were corrected by subtracting blank measurements of SJ170 WXXF peptide injected into the ITC buffer and analyzed using Origin 5.0 (MicroCal). Binding constants were calculated by fitting the integrated data to a one-site binding model.

5.2.5 Cells, Transfection, Immunofluorescence and Freeze-Etch EM

NRK cells were cultured at 37°C in DMEM, 10% FCS and 2 mM L-glutamine while undifferentiated PC12 cells were grown at 37°C in DMEM supplemented with 10% horse serum, 5% FCS and 2 mM L-glutamine. HeLa SS6 cells were grown in DME supplemented with 10% fetal calf serum and 2 mM L-glutamine at 37°C in a humidified 10 % CO_2 atmosphere. Cells were passaged onto 12 mm glass cover slips one day prior to transfection with Lipofectamine 2000. One day after transfection, cells were fixed in 2% paraformaldehyde and prepared for immunofluorescence as previously described [75]. For transferrin internalization, cells were serum starved for 1 hour, pulsed with 25 μg/ml biotin transferrin for 15 min at 37°C, washed and fixed.

For freeze-etch immunogold analysis, cells were cultured on small oriented pieces of carbon-coated glass coverslip and ruptured by sonication to generate ‘unroofed’ cell cortices

precisely as described [249]. After washing in 30 mM Hepes-KOH, pH 7.3, 70 mM potassium chloride, 5 mM magnesium chloride and 3 mM EGTA (KHMgE buffer), the cells were fixed in 2% paraformaldehyde, 0.025% glutaraldehyde in KHMgE, quenched with 50 mM ammonium chloride, 50 mM L-lysine in KHMgE, and blocked with 1% BSA in KHMgE. Coverslips were then incubated with anti-AP-2 a subunit mAb AP.6 [250] or affinity purified anti-epsin antibodies followed by anti-mouse or -rabbit antibodies conjugated to 15 nm colloidal gold. After washing in KHMgE, the membranes were fixed in 2% glutaraldehyde in KHMgE and then prepared for freeze-etch analysis [249].

APPENDIX A

DATABASE SEARCH FOR PROTEINS WITH REPEATED ENDOCYTIC MOTIFS

A.1 INTRODUCTION

A database search was conducted utilizing a PERL computer program that I wrote during my dissertation research. The search was performed using regular expression nomenclature to identify proteins containing pairs of known endocytic motifs. The database that was searched was the *Homo sapiens* proteome Release 17 from May 1, 2006 that contains 40,880 protein entries, and is freely available at the NCBI RefSeq website. Table A.1 presents the motifs used in the search. The search was conducted with pairs of protein motifs as follows: 1,1; 1,2; 1,3; 1,4; 1,5; 1,6; 2,1; etc.

Table A.1: Motifs used in computer protein database search

Number	Motif	Recognition Domain or Protein	Example Proteins
1	DP[FW]	α/β2 appendage platform subdomain of AP-2	Epsin 1, Eps15, Dab2
2	F.D.F	α appendage platform subdomain of AP-2	AP180, amphiphysin, synaptojanin 1
3	W..[FW].[DE]	α appendage sandwich subdomain of AP-2	Synaptojanin 1, Stonin 2, AAK1
4	DDGLDEAFSRLAQSRT	β2 appendage platform subdomain of AP-2	ARH, β-arrestin 1/2
5	L[LMIF][DEN][LMIF][DEN]	Clathrin HC terminal domain	AP-2, Epsin 1, Dab2, ARH, β-arrestin 1/2
6	NPF	EH domain	Epsin 1, Dab2

A.2 DATABASE SEARCH RESULTS

****
****
The pattern being searched is dp[fw].*dp[fw]

146 matches found in protein
##############################
ACCESSION = NP_055963
DEFINITION = PAS domain containing serine/threonine kinase

ACCESSION = XP_055481
DEFINITION = PREDICTED: myb-like, SWIRM and MPN domains 1

ACCESSION = NP_067051
DEFINITION = serine arginine-rich pre-mRNA splicing factor SR-A1

ACCESSION = NP_005179
DEFINITION = Cas-Br-M (murine) ecotropic retroviral transforming sequence

ACCESSION = NP_004289
DEFINITION = nucleoporin 155kDa isoform 2

ACCESSION = XP_942672
DEFINITION = PREDICTED: similar to epiplakin 1

ACCESSION = XP_496799
DEFINITION = PREDICTED: similar to Nuclear envelope pore membrane protein POM 121 (Pore membrane protein of 121 kDa) (P145)

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_006304
DEFINITION = ubiquitin specific protease 15

ACCESSION = XP_933019
DEFINITION = PREDICTED: hypothetical protein XP_933019

ACCESSION = NP_001005743
DEFINITION = numb homolog isoform 1

ACCESSION = NP_620151
DEFINITION = hypothetical protein BC014608

ACCESSION = NP_057368
DEFINITION = CCR4-NOT transcription complex, subunit 1 isoform a

ACCESSION = NP_037465
DEFINITION = epsin 1

ACCESSION = XP_946889
DEFINITION = PREDICTED: similar to myb-like, SWIRM and MPN domains 1

ACCESSION = NP_683723
DEFINITION = epsin 2 isoform a

ACCESSION = XP_379933
DEFINITION = PREDICTED: similar to Ubinuclein (Ubiquitously expressed nuclear protein) (VT4)

ACCESSION = XP_946663
DEFINITION = PREDICTED: similar to Goliath homolog precursor (RING finger protein 130) (R-goliath) isoform 4

ACCESSION = XP_062871
DEFINITION = PREDICTED: similar to Temporarily Assigned Gene name family member (tag-58)

ACCESSION = NP_490647
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 6 isoform a

ACCESSION = NP_705618
DEFINITION = nucleoporin 155kDa isoform 1

ACCESSION = NP_009099
DEFINITION = ATP-binding cassette, sub-family A member 8

ACCESSION = NP_001005744
DEFINITION = numb homolog isoform 2

ACCESSION = NP_000325
DEFINITION = sodium channel, voltage-gated, type IV, alpha

ACCESSION = NP_757366
DEFINITION = interleukin 16 isoform 2

ACCESSION = NP_476436
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 43 isoform 2

ACCESSION = NP_054858
DEFINITION = sodium channel, voltage-gated, type XI, alpha

ACCESSION = XP_944191
DEFINITION = PREDICTED: similar to Ubinuclein (Ubiquitously expressed nuclear protein) (VT4)

ACCESSION = NP_059989
DEFINITION = AT rich interactive domain 1B (SWI1-like) isoform 1

ACCESSION = NP_055889
DEFINITION = kinesin family member 1B isoform b

ACCESSION = NP_001005158
DEFINITION = Scm-like with four mbt domains 1

ACCESSION = XP_942211
DEFINITION = PREDICTED: similar to double homeobox 4c

ACCESSION = NP_055602 XP_375737
DEFINITION = DnaJ (Hsp40) homolog, subfamily C, member 6

ACCESSION = NP_733762
DEFINITION = Cas-Br-M (murine) ecotropic retroviral transforming sequence b

ACCESSION = NP_647478
DEFINITION = livin inhibitor of apoptosis isoform alpha

ACCESSION = NP_775183
DEFINITION = FXYD domain containing ion transport regulator 4

ACCESSION = NP_001005745
DEFINITION = numb homolog isoform 4

ACCESSION = NP_002968
DEFINITION = sodium channel, voltage-gated, type IX, alpha

ACCESSION = NP_877417
DEFINITION = polycystin 1-like 2 isoform b

ACCESSION = NP_055726
DEFINITION = AP2 associated kinase 1

ACCESSION = NP_056084 XP_038288
DEFINITION = zinc finger, CCHC domain containing 11 isoform b

ACCESSION = NP_001009881 XP_038288
DEFINITION = zinc finger, CCHC domain containing 11 isoform a

ACCESSION = NP_443715
DEFINITION = WD repeat domain 10 isoform 2

ACCESSION = NP_003354
DEFINITION = ubiquitin specific protease, proto-oncogene isoform a

ACCESSION = NP_006505
DEFINITION = sodium channel, voltage-gated, type X, alpha

ACCESSION = XP_944352
DEFINITION = PREDICTED: similar to Zinc finger protein 469

ACCESSION = NP_001005159
DEFINITION = Scm-like with four mbt domains 1

ACCESSION = NP_942595
DEFINITION = BMP-2 inducible kinase isoform a

ACCESSION = NP_689512

DEFINITION = kleisin beta isoform 2

ACCESSION = NP_001008566
DEFINITION = tyrosylprotein sulfotransferase 2

ACCESSION = XP_950366
DEFINITION = PREDICTED: similar to Goliath homolog precursor (RING finger protein 130) (R-goliath) isoform 6

ACCESSION = XP_933562
DEFINITION = PREDICTED: hypothetical protein XP_933562

ACCESSION = NP_115801
DEFINITION = KIAA1822 protein

ACCESSION = NP_443124
DEFINITION = polycystin 1-like 2 isoform a

ACCESSION = NP_001005526
DEFINITION = splicing factor 3b, subunit 1 isoform 2

ACCESSION = XP_938981
DEFINITION = PREDICTED: zinc and ring finger 3 isoform 3

ACCESSION = XP_377445
DEFINITION = PREDICTED: similar to double homeobox 4c

ACCESSION = XP_376679
DEFINITION = PREDICTED: hypothetical protein LOC254048

ACCESSION = NP_060570
DEFINITION = EF-hand domain (C-terminal) containing 1

ACCESSION = NP_003586
DEFINITION = tyrosylprotein sulfotransferase 2

ACCESSION = NP_877439 XP_294213 XP_353565
DEFINITION = putative binding protein 7a5

ACCESSION = NP_000756
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 7

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = NP_005485
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 6 isoform b

ACCESSION = NP_002410
DEFINITION = mitogen-activated protein kinase kinase kinase 11

ACCESSION = NP_955475
DEFINITION = ubiquitin specific protease, proto-oncogene isoform b

ACCESSION = NP_689940
DEFINITION = copine II

ACCESSION = NP_003777
DEFINITION = ATP-binding cassette, sub-family C, member 3 isoform MRP3

ACCESSION = NP_006635
DEFINITION = heat shock 105kD

ACCESSION = NP_055006
DEFINITION = sodium channel, voltage gated, type VIII, alpha

ACCESSION = NP_001972
DEFINITION = epidermal growth factor receptor pathway substrate 15

ACCESSION = XP_945788
DEFINITION = PREDICTED: similar to voltage-gated sodium channel type V alpha isoform b

ACCESSION = NP_056385
DEFINITION = autism susceptibility candidate 2

ACCESSION = NP_001028228
DEFINITION = glutamine synthetase

ACCESSION = XP_290972
DEFINITION = PREDICTED: zinc and ring finger 3 isoform 1

ACCESSION = NP_061130 NP_060076
DEFINITION = zinc finger protein 395

ACCESSION = NP_597681

DEFINITION = titin isoform novex-2

ACCESSION = NP_055779
DEFINITION = epsin 2 isoform b

ACCESSION = XP_935565
DEFINITION = PREDICTED: similar to AT rich interactive domain 1B (SWI1-like) isoform 1 isoform 1

ACCESSION = NP_624361
DEFINITION = AT rich interactive domain 1A (SWI- like) isoform b

ACCESSION = NP_005856
DEFINITION = protease, serine, 16

ACCESSION = NP_071444
DEFINITION = livin inhibitor of apoptosis isoform beta

ACCESSION = NP_060427
DEFINITION = epsin 3

ACCESSION = XP_040592
DEFINITION = PREDICTED: zinc finger protein 469

ACCESSION = NP_006805
DEFINITION = proteasome inhibitor subunit 1 isoform 1

ACCESSION = NP_057608
DEFINITION = B/K protein

ACCESSION = NP_932173
DEFINITION = voltage-gated sodium channel type V alpha isoform a

ACCESSION = NP_004066
DEFINITION = cryptochrome 1 (photolyase-like)

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_848693
DEFINITION = proteasome inhibitor subunit 1 isoform 1

ACCESSION = NP_065870 XP_371832
DEFINITION = hypothetical protein LOC57579

ACCESSION = XP_945310
DEFINITION = PREDICTED: similar to Nuclear envelope pore membrane protein POM 121 (Pore membrane protein of 121 kDa) (P145)

ACCESSION = NP_001010855 XP_375404
DEFINITION = hypothetical protein LOC146850

ACCESSION = NP_006006
DEFINITION = AT rich interactive domain 1A (SWI- like) isoform a

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_059488 NP_000767
DEFINITION = cytochrome P450, subfamily IIIA, polypeptide 4

ACCESSION = NP_003735
DEFINITION = numb homolog isoform 3

ACCESSION = NP_055656 XP_498116
DEFINITION = synaptosomal-associated protein, 91kDa homolog

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_057413 NP_056294
DEFINITION = Scm-like with four mbt domains 1

ACCESSION = NP_079303
DEFINITION = zinc finger protein 606

ACCESSION = NP_653205
DEFINITION = conserved nuclear protein NHN1

ACCESSION = NP_065731
DEFINITION = SCY1-like 1

ACCESSION = NP_036565

DEFINITION = splicing factor 3b, subunit 1 isoform 1

ACCESSION = NP_443716
DEFINITION = WD repeat domain 10 isoform 4

ACCESSION = NP_008851
DEFINITION = sodium channel, voltage-gated, type I, alpha

ACCESSION = NP_005236
DEFINITION = FAT tumor suppressor 1 precursor

ACCESSION = XP_933588
DEFINITION = PREDICTED: similar to vasoactive intestinal peptide receptor 2

ACCESSION = NP_005551
DEFINITION = laminin alpha 5

ACCESSION = NP_003894
DEFINITION = CDC16 homolog

ACCESSION = NP_067058
DEFINITION = epidermal growth factor receptor pathway substrate 15-like 1

ACCESSION = NP_060722
DEFINITION = hypothetical protein LOC55248

ACCESSION = NP_002056
DEFINITION = glutamine synthetase

ACCESSION = NP_054788
DEFINITION = SPR1 protein

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_003473
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 2

ACCESSION = XP_293293
DEFINITION = PREDICTED: similar to BMP-2 inducible protein kinase (BIKe)

ACCESSION = NP_001028216
DEFINITION = glutamine synthetase

ACCESSION = XP_942348
DEFINITION = PREDICTED: hypothetical protein XP_942348

ACCESSION = NP_065783
DEFINITION = AT rich interactive domain 1B (SWI1-like) isoform 2

ACCESSION = XP_938832
DEFINITION = PREDICTED: RW1 protein isoform 3

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_000326
DEFINITION = voltage-gated sodium channel type V alpha isoform b

ACCESSION = NP_060732
DEFINITION = WD repeat domain 10 isoform 3

ACCESSION = XP_931161
DEFINITION = PREDICTED: similar to epiplakin 1

ACCESSION = NP_001009882 XP_038288
DEFINITION = zinc finger, CCHC domain containing 11 isoform c

ACCESSION = NP_066287
DEFINITION = sodium channel, voltage-gated, type II, alpha 2

ACCESSION = XP_941787
DEFINITION = PREDICTED: similar to BMP-2 inducible protein kinase (BIKe)

ACCESSION = XP_949481
DEFINITION = PREDICTED: similar to RW1 protein isoform 8

ACCESSION = NP_005246
DEFINITION = cyclin G associated kinase

ACCESSION = NP_569713
DEFINITION = kelch-like 6

ACCESSION = NP_055180
DEFINITION = heat shock 27kDa protein 8

ACCESSION = NP_055462
DEFINITION = limkain b1

ACCESSION = NP_001026859
DEFINITION = phospholipase A2-activating protein isoform 1

ACCESSION = NP_787059
DEFINITION = AT rich interactive domain 1B (SWI1-like) isoform 3

ACCESSION = NP_443711
DEFINITION = WD repeat domain 10 isoform 1

ACCESSION = NP_005112
DEFINITION = thyroid hormone receptor associated protein 1

ACCESSION = NP_004244
DEFINITION = phospholipase A2-activating protein isoform 2

ACCESSION = NP_073731
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 43 isoform 1

ACCESSION = NP_996816 XP_117190 XP_371342 XP_372814
DEFINITION = usherin isoform B

ACCESSION = NP_476437
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 43 isoform 3

ACCESSION = NP_005849
DEFINITION = A-kinase anchor protein 8

ACCESSION = NP_071897
DEFINITION = fibrosin 1

ACCESSION = XP_942645
DEFINITION = PREDICTED: similar to Temporarily Assigned Gene name family member
(tag-58)

ACCESSION = NP_008853
DEFINITION = sodium channel, voltage-gated, type III, alpha

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The pattern being searched is dp[fw].*f.d.f

66 matches found in protein
##########
ACCESSION = XP_932418
DEFINITION = PREDICTED: similar to ovo-like 2 isoform A

ACCESSION = NP_579899
DEFINITION = myoferlin isoform b

ACCESSION = NP_612149
DEFINITION = ataxia telangiectasia mutated protein isoform 2

ACCESSION = XP_942672
DEFINITION = PREDICTED: similar to epiplakin 1

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_000229
DEFINITION = voltage-gated potassium channel, subfamily H, member 2 isoform a

ACCESSION = NP_003465
DEFINITION = ADAM metallopeptidase domain 12 isoform 1 preproprotein

ACCESSION = NP_065816
DEFINITION = retinoblastoma-associated factor 600

ACCESSION = NP_071343
DEFINITION = abhydrolase domain containing 4

ACCESSION = NP_006109
DEFINITION = HCLS1 associated protein X-1 isoform a

ACCESSION = NP_490647
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 6 isoform a

ACCESSION = NP_476436
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 43 isoform 2

ACCESSION = NP_171608
DEFINITION = hypothetical protein LOC84752

ACCESSION = NP_000042
DEFINITION = ataxia telangiectasia mutated protein isoform 1

ACCESSION = NP_015628
DEFINITION = ankyrin-like protein 1

ACCESSION = NP_067673
DEFINITION = ADAM metallopeptidase domain 12 isoform 2 preproprotein

ACCESSION = NP_000519
DEFINITION = mannosidase, alpha, class 2B, member 1 precursor

ACCESSION = XP_941968
DEFINITION = PREDICTED: similar to jumonji domain containing 2D

ACCESSION = XP_943991
DEFINITION = PREDICTED: similar to syntaxin binding protein 5-like

ACCESSION = NP_776152
DEFINITION = PDZ domain containing 8

ACCESSION = NP_057692
DEFINITION = armadillo repeat containing, X-linked 1

ACCESSION = NP_001005526
DEFINITION = splicing factor 3b, subunit 1 isoform 2

ACCESSION = NP_055660
DEFINITION = Sac domain-containing inositol phosphatase 3

ACCESSION = NP_037534
DEFINITION = fatty acid desaturase 1

ACCESSION = NP_002482
DEFINITION = NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 3, 12kDa

ACCESSION = NP_653283
DEFINITION = likley ortholog of mouse schlafen 10

ACCESSION = NP_005485
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 6 isoform b

ACCESSION = NP_065843
DEFINITION = arylacetamide deacetylase-like 1

ACCESSION = NP_078898
DEFINITION = hypothetical protein LOC79675

ACCESSION = NP_001972
DEFINITION = epidermal growth factor receptor pathway substrate 15

ACCESSION = NP_060466
DEFINITION = de-etiolated 1

ACCESSION = NP_525023
DEFINITION = ATP-binding cassette, sub-family A, member 6

ACCESSION = NP_000760
DEFINITION = cytochrome P450, family 2, subfamily C, polypeptide 19

ACCESSION = NP_005382
DEFINITION = pyruvate dehydrogenase kinase, isoenzyme 3

ACCESSION = NP_004845
DEFINITION = HNK-1 sulfotransferase

ACCESSION = NP_055434
DEFINITION = glutamate receptor KA1 precursor

ACCESSION = NP_002967
DEFINITION = sodium channel, voltage-gated, type VII, alpha

ACCESSION = XP_372429
DEFINITION = PREDICTED: similar to jumonji domain containing 2D

ACCESSION = NP_005430
DEFINITION = myeloid leukemia factor 2

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_056271
DEFINITION = cofactor of BRCA1

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_059488 NP_000767
DEFINITION = cytochrome P450, subfamily IIIA, polypeptide 4

ACCESSION = XP_945298
DEFINITION = PREDICTED: similar to ovo-like 2 isoform A

ACCESSION = NP_055656 XP_498116
DEFINITION = synaptosomal-associated protein, 91kDa homolog

ACCESSION = NP_005751
DEFINITION = CCAAT/enhancer binding protein zeta

ACCESSION = NP_004527
DEFINITION = baculoviral IAP repeat-containing 1

ACCESSION = NP_001027
DEFINITION = ryanodine receptor 3

ACCESSION = NP_067058
DEFINITION = epidermal growth factor receptor pathway substrate 15-like 1

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_038479
DEFINITION = myoferlin isoform a

ACCESSION = XP_944474
DEFINITION = PREDICTED: similar to cofactor of BRCA1

ACCESSION = NP_065201
DEFINITION = sorting nexin 14 isoform b

ACCESSION = XP_931161
DEFINITION = PREDICTED: similar to epiplakin 1

ACCESSION = NP_722523
DEFINITION = sorting nexin 14 isoform a

ACCESSION = NP_955452 NP_054844
DEFINITION = DNA polymerase theta

ACCESSION = XP_045911
DEFINITION = PREDICTED: syntaxin binding protein 5-like

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_064450 XP_371352
DEFINITION = formin 2

ACCESSION = NP_001026859
DEFINITION = phospholipase A2-activating protein isoform 1

ACCESSION = XP_945884
DEFINITION = PREDICTED: similar to Serine-protein kinase ATM (Ataxia telangiectasia mutated) (A-T, mutated)

ACCESSION = NP_004244
DEFINITION = phospholipase A2-activating protein isoform 2

ACCESSION = NP_073731
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 43 isoform 1

ACCESSION = NP_996816 XP_117190 XP_371342 XP_372814
DEFINITION = usherin isoform B

ACCESSION = NP_476437
DEFINITION = cytochrome P450, family 3, subfamily A, polypeptide 43 isoform 3

ACCESSION = NP_004811
DEFINITION = cytochrome P450, family 7, subfamily B, polypeptide 1

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The pattern being searched is dp[fw].*w..[fw].[de]

55 matches found in protein

##############################
ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = XP_093087
DEFINITION = PREDICTED: similar to family with sequence similarity 48, member A isoform b

ACCESSION = NP_006304
DEFINITION = ubiquitin specific protease 15

ACCESSION = NP_002582
DEFINITION = cytosolic phosphoenolpyruvate carboxykinase 1

ACCESSION = NP_079284
DEFINITION = thrombospondin repeat containing 1 isoform 2

ACCESSION = NP_060087
DEFINITION = notch1 preproprotein

ACCESSION = NP_037513
DEFINITION = thyrotropin-releasing hormone degrading enzyme

ACCESSION = NP_004196
DEFINITION = ubiquitin specific protease 2 isoform a

ACCESSION = NP_115946
DEFINITION = ubiquitin specific protease 38

ACCESSION = XP_056455
DEFINITION = PREDICTED: Melanoma associated gene isoform 1

ACCESSION = NP_647478
DEFINITION = livin inhibitor of apoptosis isoform alpha

ACCESSION = NP_851607
DEFINITION = SCY1-like 3 isoform 2

ACCESSION = NP_003354
DEFINITION = ubiquitin specific protease, proto-oncogene isoform a

ACCESSION = XP_293352
DEFINITION = PREDICTED: similar to family with sequence similarity 48, member A isoform a

ACCESSION = NP_689512
DEFINITION = kleisin beta isoform 2

ACCESSION = NP_004642
DEFINITION = ubiquitin specific protease 11

ACCESSION = XP_947700
DEFINITION = PREDICTED: similar to family with sequence similarity 48, member A isoform b

ACCESSION = XP_933562
DEFINITION = PREDICTED: hypothetical protein XP_933562

ACCESSION = NP_004548
DEFINITION = notch4 preproprotein

ACCESSION = NP_006668 XP_496642
DEFINITION = ubiquitin specific protease 19

ACCESSION = NP_061905
DEFINITION = thrombospondin repeat containing 1 isoform 1

ACCESSION = NP_002410
DEFINITION = mitogen-activated protein kinase kinase kinase 11

ACCESSION = NP_955475
DEFINITION = ubiquitin specific protease, proto-oncogene isoform b

ACCESSION = NP_064581
DEFINITION = XPA binding protein 2

ACCESSION = NP_065769 XP_049683
DEFINITION = ubiquitin specific protease 31

ACCESSION = XP_933202
DEFINITION = PREDICTED: hypothetical protein XP_933202

ACCESSION = NP_068570
DEFINITION = interleukin 21 receptor precursor

ACCESSION = NP_036397
DEFINITION = chromosome 22 open reading frame 3

ACCESSION = NP_006528
DEFINITION = ubiquitin specific protease 3

ACCESSION = NP_071444
DEFINITION = livin inhibitor of apoptosis isoform beta

ACCESSION = NP_001010870 XP_166443
DEFINITION = tudor domain containing 6

ACCESSION = NP_851564
DEFINITION = interleukin 21 receptor precursor

ACCESSION = NP_002842
DEFINITION = protein tyrosine phosphatase, receptor-type, Z polypeptide 1

ACCESSION = NP_851565
DEFINITION = interleukin 21 receptor precursor

ACCESSION = NP_004066
DEFINITION = cryptochrome 1 (photolyase-like)

ACCESSION = NP_689947
DEFINITION = hypothetical protein LOC221477

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_114128
DEFINITION = RALBP1 associated Eps domain containing 1

ACCESSION = NP_891550
DEFINITION = ADAM metallopeptidase with thrombospondin type 1 motif, 9 preproprotein

ACCESSION = NP_004554
DEFINITION = mitochondrial phosphoenolpyruvate carboxykinase 2 isoform 1 precursor

ACCESSION = NP_008860
DEFINITION = superkiller viralicidic activity 2-like homolog

ACCESSION = NP_001027
DEFINITION = ryanodine receptor 3

ACCESSION = NP_005693
DEFINITION = Era G-protein-like 1

ACCESSION = NP_065156
DEFINITION = SCY1-like 3 isoform 1

ACCESSION = XP_933588
DEFINITION = PREDICTED: similar to vasoactive intestinal peptide receptor 2

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_000123
DEFINITION = coagulation factor VIII isoform a precursor

ACCESSION = NP_037434
DEFINITION = elongation factor-2 kinase

ACCESSION = NP_002323
DEFINITION = low density lipoprotein-related protein 1

ACCESSION = XP_944058
DEFINITION = PREDICTED: hypothetical protein XP_944058

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_005246
DEFINITION = cyclin G associated kinase

ACCESSION = XP_941879
DEFINITION = PREDICTED: similar to peroxidasin isoform 7

ACCESSION = NP_061027
DEFINITION = low density lipoprotein-related protein 1B

ACCESSION = NP_741994
DEFINITION = ubiquitin specific protease 2 isoform b

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****
The pattern being searched is dp[fw].*DDGLDEAFSRLAQSRT

No matches found

****
****
The pattern being searched is dp[fw].*L[LMIF][DEN][LMIF][DEN]

75 matches found in protein
##############################
ACCESSION = NP_079146
DEFINITION = DEP domain containing 2 isoform a

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_775080
DEFINITION = zonadhesin isoform 4

ACCESSION = NP_057368
DEFINITION = CCR4-NOT transcription complex, subunit 1 isoform a

ACCESSION = NP_003377
DEFINITION = zonadhesin isoform 3

ACCESSION = NP_055860 XP_290471
DEFINITION = KIAA0261

ACCESSION = NP_003697
DEFINITION = phospholipase A2, group IVC

ACCESSION = XP_379933
DEFINITION = PREDICTED: similar to Ubinuclein (Ubiquitously expressed nuclear protein) (VT4)

ACCESSION = NP_001026971
DEFINITION = LIM domain kinase 2 isoform 1

ACCESSION = NP_055751 XP_376503
DEFINITION = ectonucleotide pyrophosphatase/phosphodiesterase 4 (putative function)

ACCESSION = XP_944191
DEFINITION = PREDICTED: similar to Ubinuclein (Ubiquitously expressed nuclear protein) (VT4)

ACCESSION = NP_059447
DEFINITION = major vault protein

ACCESSION = NP_009094
DEFINITION = solute carrier family 14 (urea transporter), member 2

ACCESSION = NP_085124
DEFINITION = dicer1

ACCESSION = NP_001003935
DEFINITION = poly (ADP-ribose) polymerase family, member 3 isoform b

ACCESSION = XP_946300
DEFINITION = PREDICTED: similar to FRAS1-related extracellular matrix protein 2 precursor (ECM3 homolog)

ACCESSION = XP_934977
DEFINITION = PREDICTED: FRAS1 related extracellular matrix protein 2

ACCESSION = XP_931292
DEFINITION = PREDICTED: FAT tumor suppressor homolog 3

ACCESSION = NP_877417
DEFINITION = polycystin 1-like 2 isoform b

ACCESSION = NP_443715
DEFINITION = WD repeat domain 10 isoform 2

ACCESSION = NP_775082
DEFINITION = zonadhesin isoform 6

ACCESSION = NP_055935
DEFINITION = ALMS1

ACCESSION = NP_004227
DEFINITION = COP9 constitutive photomorphogenic homolog subunit 2

ACCESSION = NP_005106
DEFINITION = major vault protein

ACCESSION = NP_071373 XP_371779
DEFINITION = transposon-derived Buster3 transposase-like

ACCESSION = NP_443124
DEFINITION = polycystin 1-like 2 isoform a

ACCESSION = NP_003485
DEFINITION = dysferlin

ACCESSION = NP_005436
DEFINITION = chondroitin sulfate proteoglycan 6 (bamacan)

ACCESSION = XP_376679
DEFINITION = PREDICTED: hypothetical protein LOC254048

ACCESSION = NP_149132 XP_027237
DEFINITION = mitogen-activated protein kinase kinase kinase 9

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = NP_060241
DEFINITION = PX domain containing serine/threonine kinase

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = NP_001004051
DEFINITION = G protein-coupled receptor associated sorting protein 2

ACCESSION = NP_078898
DEFINITION = hypothetical protein LOC79675

ACCESSION = NP_071762
DEFINITION = semaphorin B

ACCESSION = NP_003728
DEFINITION = dachsous 1 precursor

ACCESSION = NP_058632
DEFINITION = ubinuclein 1

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = NP_114068
DEFINITION = neighbor of BRCA1 gene 1

ACCESSION = NP_037373
DEFINITION = formin homology 2 domain containing 1

ACCESSION = NP_005137
DEFINITION = squamous cell carcinoma antigen recognized by T cells 1

ACCESSION = NP_055249
DEFINITION = NADPH dependent diflavin oxidoreductase 1

ACCESSION = NP_803187
DEFINITION = dicer1

ACCESSION = NP_775079
DEFINITION = zonadhesin isoform 2

ACCESSION = NP_932173
DEFINITION = voltage-gated sodium channel type V alpha isoform a

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_733751
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 3 isoform 2

ACCESSION = NP_114128
DEFINITION = RALBP1 associated Eps domain containing 1

ACCESSION = NP_775081
DEFINITION = zonadhesin isoform 5

ACCESSION = NP_075463
DEFINITION = hypothetical protein CG003

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_443716
DEFINITION = WD repeat domain 10 isoform 4

ACCESSION = XP_945361
DEFINITION = PREDICTED: similar to NADPH dependent diflavin oxidoreductase 1

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_003473
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 2

ACCESSION = NP_005890
DEFINITION = neighbor of BRCA1 gene 1

ACCESSION = NP_115495
DEFINITION = very large G-protein coupled receptor 1

ACCESSION = NP_002437
DEFINITION = mitogen-activated protein kinase kinase kinase 10

ACCESSION = NP_000326
DEFINITION = voltage-gated sodium channel type V alpha isoform b

ACCESSION = NP_060732
DEFINITION = WD repeat domain 10 isoform 3

ACCESSION = NP_067053
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 3 isoform 1

ACCESSION = NP_955452 NP_054844
DEFINITION = DNA polymerase theta

ACCESSION = XP_093839
DEFINITION = PREDICTED: hypothetical protein LOC23045 isoform 1

ACCESSION = NP_005476
DEFINITION = poly (ADP-ribose) polymerase family, member 3 isoform b

ACCESSION = NP_001003931
DEFINITION = poly (ADP-ribose) polymerase family, member 3 isoform a

ACCESSION = NP_612446
DEFINITION = G protein-coupled receptor associated sorting protein 2

ACCESSION = NP_937762
DEFINITION = laminin alpha 3 subunit isoform 1

ACCESSION = XP_946679
DEFINITION = PREDICTED: similar to CG32045-PB, isoform B isoform 5

ACCESSION = NP_443711
DEFINITION = WD repeat domain 10 isoform 1

ACCESSION = XP_941631
DEFINITION = PREDICTED: similar to fat3

ACCESSION = NP_001026877
DEFINITION = hypothetical protein LOC55239 isoform 1

ACCESSION = NP_114064
DEFINITION = neighbor of BRCA1 gene 1

ACCESSION = NP_775078
DEFINITION = zonadhesin isoform 1

ACCESSION = NP_008965
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 4

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The pattern being searched is dp[fw].*npf

89 matches found in protein
##########
ACCESSION = NP_003613
DEFINITION = PTPRF interacting protein binding protein 1 isoform 1

ACCESSION = NP_055963
DEFINITION = PAS domain containing serine/threonine kinase

ACCESSION = NP_612149
DEFINITION = ataxia telangiectasia mutated protein isoform 2

ACCESSION = XP_945150
DEFINITION = PREDICTED: similar to human immunodeficiency virus type I enhancer binding protein 3

ACCESSION = NP_001005743
DEFINITION = numb homolog isoform 1

ACCESSION = NP_037465
DEFINITION = epsin 1

ACCESSION = NP_683723
DEFINITION = epsin 2 isoform a

ACCESSION = NP_940961 XP_351515
DEFINITION = hypothetical protein LOC375307

ACCESSION = NP_001004454 XP_071150
DEFINITION = olfactory receptor, family 1, subfamily L, member 8

ACCESSION = NP_001002847
DEFINITION = decreased expression in renal and prostate

ACCESSION = NP_996535
DEFINITION = myelin oligodendrocyte glycoprotein isoform alpha2 precursor

ACCESSION = NP_060043
DEFINITION = proprotein convertase subtilisin/kexin type 4

ACCESSION = XP_372255
DEFINITION = PREDICTED: similar to CG4768-PA

ACCESSION = NP_001208
DEFINITION = carbonic anhydrase XI precursor

ACCESSION = XP_942656
DEFINITION = PREDICTED: hypothetical protein XP_942656

ACCESSION = NP_001005744
DEFINITION = numb homolog isoform 2

ACCESSION = NP_000454
DEFINITION = UDP glycosyltransferase 1 family, polypeptide A1 precursor

ACCESSION = NP_000042
DEFINITION = ataxia telangiectasia mutated protein isoform 1

ACCESSION = NP_054789
DEFINITION = STG protein

ACCESSION = XP_931423
DEFINITION = PREDICTED: hypothetical protein XP_931423

ACCESSION = XP_942211
DEFINITION = PREDICTED: similar to double homeobox 4c

ACCESSION = NP_001005745
DEFINITION = numb homolog isoform 4

ACCESSION = XP_086287
DEFINITION = PREDICTED: similar to protein tyrosine phosphatase, receptor type, V

ACCESSION = NP_113686
DEFINITION = guanine nucleotide binding protein-gamma transducing activity polypeptide 2

ACCESSION = NP_006067
DEFINITION = HIV-1 Rev-binding protein-like protein

ACCESSION = NP_001005736
DEFINITION = lysosomal trafficking regulator isoform 2

ACCESSION = NP_955525
DEFINITION = 5-hydroxytryptamine (serotonin) receptor 4 isoform g

ACCESSION = NP_001501
DEFINITION = glutamate receptor, ionotropic, delta 2

ACCESSION = NP_620278
DEFINITION = TBP-associated factor 1 isoform 2

ACCESSION = NP_000861
DEFINITION = 5-hydroxytryptamine (serotonin) receptor 4 isoform b

ACCESSION = NP_115801
DEFINITION = KIAA1822 protein

ACCESSION = XP_946113
DEFINITION = PREDICTED: similar to protein tyrosine phosphatase, receptor type, V

ACCESSION = NP_001004737
DEFINITION = olfactory receptor, family 5, subfamily K, member 2

ACCESSION = XP_377445
DEFINITION = PREDICTED: similar to double homeobox 4c

ACCESSION = NP_003546
DEFINITION = olfactory receptor, family 1, subfamily G, member 1

ACCESSION = NP_000027
DEFINITION = adenosine monophosphate deaminase 1 (isoform M)

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = NP_002410
DEFINITION = mitogen-activated protein kinase kinase kinase 11

ACCESSION = NP_689940
DEFINITION = copine II

ACCESSION = NP_001805
DEFINITION = cathepsin C isoform a preproprotein

ACCESSION = NP_009097
DEFINITION = phosphatidylinositol-binding clathrin assembly protein isoform 1

ACCESSION = NP_058632
DEFINITION = ubinuclein 1

ACCESSION = NP_060115
DEFINITION = hypothetical protein LOC54801

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = NP_055779
DEFINITION = epsin 2 isoform b

ACCESSION = NP_060274
DEFINITION = decreased expression in renal and prostate

ACCESSION = NP_060427
DEFINITION = epsin 3

ACCESSION = NP_061753
DEFINITION = protocadherin beta 10 precursor

ACCESSION = NP_002967
DEFINITION = sodium channel, voltage-gated, type VII, alpha

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_733751
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 3 isoform 2

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_079022
DEFINITION = hypothetical protein LOC79802

ACCESSION = NP_000183
DEFINITION = T-box 5 isoform 1

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_003735
DEFINITION = numb homolog isoform 3

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_055719
DEFINITION = RAB11 family interacting protein 2 (class I)

ACCESSION = NP_955372 XP_351142
DEFINITION = hypothetical protein LOC374819

ACCESSION = NP_003390
DEFINITION = X-prolyl aminopeptidase 2, membrane-bound

ACCESSION = NP_000072
DEFINITION = lysosomal trafficking regulator isoform 1

ACCESSION = XP_066058
DEFINITION = PREDICTED: hypothetical protein LOC128611

ACCESSION = NP_996532
DEFINITION = myelin oligodendrocyte glycoprotein isoform alpha1 precursor

ACCESSION = NP_064563
DEFINITION = carbonic anhydrase X

ACCESSION = NP_061992
DEFINITION = protocadherin beta 9 precursor

ACCESSION = NP_055790
DEFINITION = microtubule associated serine/threonine kinase 1

ACCESSION = NP_004248
DEFINITION = TGF beta receptor associated protein -1

ACCESSION = NP_542448
DEFINITION = T-box 5 isoform 3

ACCESSION = NP_004747
DEFINITION = numb homolog (Drosophila)-like

ACCESSION = XP_950074
DEFINITION = PREDICTED: hypothetical protein XP_950074

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_067053
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 3 isoform 1

ACCESSION = NP_542449
DEFINITION = T-box 5 isoform 2

ACCESSION = NP_955452 NP_054844
DEFINITION = DNA polymerase theta

ACCESSION = NP_060958
DEFINITION = T-box 4

ACCESSION = NP_000341
DEFINITION = ATP-binding cassette, sub-family A member 4

ACCESSION = XP_940860
DEFINITION = PREDICTED: hypothetical protein LOC54801

ACCESSION = XP_941802
DEFINITION = PREDICTED: similar to CG4768-PA

ACCESSION = NP_060225
DEFINITION = NOL1/NOP2/Sun domain family 2 protein

ACCESSION = NP_066267
DEFINITION = ankyrin 3 isoform 1

ACCESSION = NP_722516
DEFINITION = TBP-associated factor RNA polymerase 1-like

ACCESSION = XP_945884
DEFINITION = PREDICTED: similar to Serine-protein kinase ATM (Ataxia telangiectasia mutated) (A-T, mutated)

ACCESSION = NP_659490
DEFINITION = hypothetical protein LOC143630

ACCESSION = NP_059509
DEFINITION = ubiquilin 3

ACCESSION = NP_919268 XP_089143
DEFINITION = spindle assembly abnormal protein 6

ACCESSION = XP_946024
DEFINITION = PREDICTED: similar to fibrillarin

ACCESSION = NP_852259
DEFINITION = T-box 5 isoform 1

ACCESSION = NP_001008229
DEFINITION = myelin oligodendrocyte glycoprotein isoform alpha3 precursor

ACCESSION = NP_004597
DEFINITION = TBP-associated factor 1 isoform 1

****
****
The pattern being searched is f.d.f.*dp[fw]

83 matches found in protein
##############################
ACCESSION = NP_543009
DEFINITION = G protein-coupled receptor 78

ACCESSION = NP_005582
DEFINITION = meiotic recombination 11 homolog A isoform 1

ACCESSION = NP_079146
DEFINITION = DEP domain containing 2 isoform a

ACCESSION = NP_004289
DEFINITION = nucleoporin 155kDa isoform 2

ACCESSION = NP_877963
DEFINITION = phospholipase C gamma 1 isoform b

ACCESSION = NP_005622
DEFINITION = smoothened

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_001626
DEFINITION = amphiphysin isoform 1

ACCESSION = NP_000435
DEFINITION = X-linked phosphate regulating endopeptidase homolog

ACCESSION = NP_075561
DEFINITION = hypothetical protein LOC65250

ACCESSION = NP_065997 XP_497076
DEFINITION = hypothetical protein LOC57706 isoform 1

ACCESSION = NP_001082
DEFINITION = amiloride binding protein 1 precursor

ACCESSION = NP_705618
DEFINITION = nucleoporin 155kDa isoform 1

ACCESSION = NP_009099
DEFINITION = ATP-binding cassette, sub-family A member 8

ACCESSION = XP_943763
DEFINITION = PREDICTED: hypothetical protein LOC348487

ACCESSION = NP_005329
DEFINITION = huntingtin interacting protein 1

ACCESSION = NP_001008536 XP_060104
DEFINITION = trichohyalin-like 1

ACCESSION = NP_055889
DEFINITION = kinesin family member 1B isoform b

ACCESSION = NP_000042
DEFINITION = ataxia telangiectasia mutated protein isoform 1

ACCESSION = NP_005581
DEFINITION = meiotic recombination 11 homolog A isoform 2

ACCESSION = NP_085124
DEFINITION = dicer1

ACCESSION = NP_652761
DEFINITION = A-kinase anchor protein 11 isoform 2

ACCESSION = XP_371501
DEFINITION = PREDICTED: hypothetical protein LOC200424

ACCESSION = XP_944352
DEFINITION = PREDICTED: similar to Zinc finger protein 469

ACCESSION = NP_703143
DEFINITION = G protein-coupled receptor 26

ACCESSION = XP_943674
DEFINITION = PREDICTED: similar to pleckstrin homology domain containing, family M (with RUN domain) member 1

ACCESSION = NP_036586
DEFINITION = T-cell lymphoma invasion and metastasis 2 isoform a

ACCESSION = NP_005207
DEFINITION = dolichyl-diphosphooligosaccharide-protein glycosyltransferase

ACCESSION = NP_071373 XP_371779
DEFINITION = transposon-derived Buster3 transposase-like

ACCESSION = NP_061111
DEFINITION = carbohydrate (chondroitin 4) sulfotransferase 12

ACCESSION = NP_690867
DEFINITION = kelch repeat and BTB (POZ) domain-containing 6

ACCESSION = NP_871626
DEFINITION = protein phosphatase 2, regulatory subunit B", alpha isoform 2

ACCESSION = NP_919224
DEFINITION = otoferlin isoform a

ACCESSION = NP_004377
DEFINITION = chondroitin sulfate proteoglycan 3 (neurocan)

ACCESSION = NP_055146
DEFINITION = solute carrier family 7, (cationic amino acid transporter, y+ system) member 11

ACCESSION = NP_689522
DEFINITION = phosphoinositide-3-kinase adaptor protein 1

ACCESSION = NP_006039
DEFINITION = ubiquitination factor E4B

ACCESSION = NP_001008226
DEFINITION = CCR4-NOT transcription complex, subunit 4 isoform b

ACCESSION = NP_957720
DEFINITION = membrane-bound transcription factor site-1 protease isoform 2 preproprotein

ACCESSION = NP_006635
DEFINITION = heat shock 105kD

ACCESSION = NP_647477
DEFINITION = amphiphysin isoform 2

ACCESSION = NP_061859
DEFINITION = SH3 domain and tetratricopeptide repeats 1

ACCESSION = NP_001028228
DEFINITION = glutamine synthetase

ACCESSION = XP_941991
DEFINITION = PREDICTED: similar to jumonji domain containing 2D

ACCESSION = NP_055139
DEFINITION = alpha-methylacyl-CoA racemase isoform 1

ACCESSION = XP_947119
DEFINITION = PREDICTED: similar to CXXC finger 6

ACCESSION = NP_002709
DEFINITION = protein phosphatase 2, regulatory subunit B", alpha isoform 1

ACCESSION = NP_005586
DEFINITION = nuclear factor I/A

ACCESSION = XP_040592
DEFINITION = PREDICTED: zinc finger protein 469

ACCESSION = NP_065871
DEFINITION = PREX1 protein

ACCESSION = NP_803187
DEFINITION = dicer1

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = XP_371542
DEFINITION = PREDICTED: RW1 protein isoform 1

ACCESSION = NP_002651
DEFINITION = phospholipase C gamma 1 isoform a

ACCESSION = NP_037448
DEFINITION = CCR4-NOT transcription complex, subunit 4 isoform a

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_055656 XP_498116
DEFINITION = synaptosomal-associated protein, 91kDa homolog

ACCESSION = NP_057114
DEFINITION = tetratricopeptide repeat domain 15

ACCESSION = XP_945025
DEFINITION = PREDICTED: similar to RW1 protein isoform 6

ACCESSION = NP_525021
DEFINITION = ATP-binding cassette, sub-family A, member 10

ACCESSION = NP_055731
DEFINITION = lemur tyrosine kinase 2

ACCESSION = NP_067058
DEFINITION = epidermal growth factor receptor pathway substrate 15-like 1

ACCESSION = NP_002056
DEFINITION = glutamine synthetase

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_001028216
DEFINITION = glutamine synthetase

ACCESSION = XP_931595
DEFINITION = PREDICTED: similar to jumonji domain containing 2D

ACCESSION = NP_056234
DEFINITION = adlican

ACCESSION = NP_000341
DEFINITION = ATP-binding cassette, sub-family A member 4

ACCESSION = NP_002652
DEFINITION = phospholipase C, gamma 2 (phosphatidylinositol-specific)

ACCESSION = NP_057332
DEFINITION = A-kinase anchor protein 11 isoform 1

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_002363
DEFINITION = mannosidase, alpha, class 2A, member 1

ACCESSION = NP_001026859
DEFINITION = phospholipase A2-activating protein isoform 1

ACCESSION = XP_290944
DEFINITION = PREDICTED: pleckstrin homology domain containing, family M (with RUN domain) member 2

ACCESSION = NP_055753
DEFINITION = mondoA

ACCESSION = NP_065121
DEFINITION = dystonin isoform 1eB precursor

ACCESSION = NP_061948
DEFINITION = UDP glycosyltransferase 1 family, polypeptide A10 precursor

ACCESSION = XP_945884
DEFINITION = PREDICTED: similar to Serine-protein kinase ATM (Ataxia telangiectasia mutated) (A-T, mutated)

ACCESSION = NP_003905
DEFINITION = cyclin A1

ACCESSION = NP_004244
DEFINITION = phospholipase A2-activating protein isoform 2

ACCESSION = NP_996816 XP_117190 XP_371342 XP_372814
DEFINITION = usherin isoform B

ACCESSION = NP_115514
DEFINITION = kelch repeat and BTB (POZ) domain containing 7

ACCESSION = XP_113971
DEFINITION = PREDICTED: similar to zinc finger and BTB domain containing 36

****
****
The pattern being searched is f.d.f.*f.d.f

68 matches found in protein
##########
ACCESSION = NP_065965 XP_290768
DEFINITION = chromosome 17 open reading frame 27

ACCESSION = NP_001017372
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = XP_947086
DEFINITION = PREDICTED: similar to stereocilin

ACCESSION = NP_000755
DEFINITION = cytochrome P450, family 2, subfamily A, polypeptide 7 isoform 1

ACCESSION = NP_055595
DEFINITION = cullin 7

ACCESSION = NP_001295
DEFINITION = carboxypeptidase D precursor

ACCESSION = NP_937756
DEFINITION = CUB and Sushi multiple domains 3 isoform 1

ACCESSION = XP_943763
DEFINITION = PREDICTED: hypothetical protein LOC348487

ACCESSION = NP_000042
DEFINITION = ataxia telangiectasia mutated protein isoform 1

ACCESSION = NP_694857
DEFINITION = solute carrier family 22 member 1 isoform b

ACCESSION = NP_612144
DEFINITION = deltex 3-like

ACCESSION = NP_003048
DEFINITION = solute carrier family 22 member 1 isoform a

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_036252
DEFINITION = CD2-associated protein

ACCESSION = NP_085079
DEFINITION = cytochrome P450, family 2, subfamily A, polypeptide 7 isoform 2

ACCESSION = NP_000753
DEFINITION = cytochrome P450, family 2, subfamily A, polypeptide 6

ACCESSION = NP_150094
DEFINITION = CUB and Sushi multiple domains 1

ACCESSION = NP_000757
DEFINITION = cytochrome P450, family 2, subfamily A, polypeptide 13

ACCESSION = NP_477513
DEFINITION = monoacylglycerol O-acyltransferase 1

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = NP_775774
DEFINITION = hypothetical protein LOC146779

ACCESSION = NP_955456
DEFINITION = WW domain containing E3 ubiquitin protein ligase 2 isoform 2

ACCESSION = NP_054750
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_008944
DEFINITION = WW domain containing E3 ubiquitin protein ligase 1

ACCESSION = NP_001026
DEFINITION = ryanodine receptor 2

ACCESSION = NP_005752
DEFINITION = plexin C1

ACCESSION = NP_001015045 XP_371697 XP_376328
DEFINITION = family with sequence similarity 13, member A1 isoform b

ACCESSION = NP_060466
DEFINITION = de-etiolated 1

ACCESSION = XP_943543
DEFINITION = PREDICTED: similar to zinc finger and BTB domain containing 36

ACCESSION = XP_941345
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_060828
DEFINITION = ATP-binding cassette, sub-family F (GCN20), member 3

ACCESSION = XP_940697
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_037506
DEFINITION = programmed cell death 6 interacting protein

ACCESSION = NP_005303
DEFINITION = guanine nucleotide-releasing factor 2 isoform a

ACCESSION = NP_001019837
DEFINITION = SH3-domain kinase binding protein 1 isoform b

ACCESSION = NP_937757
DEFINITION = CUB and Sushi multiple domains 3 isoform 2

ACCESSION = NP_008945
DEFINITION = WW domain containing E3 ubiquitin protein ligase 2 isoform 1

ACCESSION = NP_065708
DEFINITION = zinc finger protein 304

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = NP_001032
DEFINITION = sucrase-isomaltase (alpha-glucosidase)

ACCESSION = XP_944522
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_055434
DEFINITION = glutamate receptor KA1 precursor

ACCESSION = NP_444267
DEFINITION = UDP glycosyltransferase 2 family, polypeptide B28

ACCESSION = NP_061720
DEFINITION = axonemal dynein heavy chain 7

ACCESSION = NP_001019820
DEFINITION = calnexin precursor

ACCESSION = NP_055656 XP_498116
DEFINITION = synaptosomal-associated protein, 91kDa homolog

ACCESSION = NP_001027
DEFINITION = ryanodine receptor 3

ACCESSION = NP_001596
DEFINITION = alanyl-tRNA synthetase

ACCESSION = NP_001737
DEFINITION = calnexin precursor

ACCESSION = NP_036460
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 9

ACCESSION = NP_005115
DEFINITION = nucleoporin 153kDa

ACCESSION = NP_055698 XP_376328
DEFINITION = family with sequence similarity 13, member A1 isoform a

ACCESSION = NP_612152
DEFINITION = polycystin-1L1

ACCESSION = XP_931230
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_006155
DEFINITION = nuclear factor (erythroid-derived 2)-like 2

ACCESSION = NP_001037
DEFINITION = solute carrier family 12 (sodium/potassium/chloride transporters), member 2

ACCESSION = NP_002290
DEFINITION = lactase-phlorizin hydrolase preproprotein

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_055904
DEFINITION = p53-associated parkin-like cytoplasmic protein

ACCESSION = NP_714544
DEFINITION = stereocilin

ACCESSION = NP_055753
DEFINITION = mondoA

ACCESSION = NP_061948
DEFINITION = UDP glycosyltransferase 1 family, polypeptide A10 precursor

ACCESSION = NP_000763
DEFINITION = cytochrome P450, family 2, subfamily C, polypeptide 18

ACCESSION = XP_945884
DEFINITION = PREDICTED: similar to Serine-protein kinase ATM (Ataxia telangiectasia mutated) (A-T, mutated)

ACCESSION = NP_114098
DEFINITION = SH3-domain kinase binding protein 1 isoform a

ACCESSION = NP_941372
DEFINITION = guanine nucleotide-releasing factor 2 isoform b

ACCESSION = XP_113971
DEFINITION = PREDICTED: similar to zinc finger and BTB domain containing 36

ACCESSION = NP_443132
DEFINITION = CUB and Sushi multiple domains 3 isoform 3

****
****
The pattern being searched is f.d.f.*w..[fw].[de]

66 matches found in protein
##############################
ACCESSION = XP_946284
DEFINITION = PREDICTED: similar to ataxia telangiectasia and Rad3 related protein

ACCESSION = NP_001017372
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = XP_947086
DEFINITION = PREDICTED: similar to stereocilin

ACCESSION = XP_951183
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 5

ACCESSION = XP_497655
DEFINITION = PREDICTED: similar to PRAME family member 8

ACCESSION = NP_055595

DEFINITION = cullin 7

ACCESSION = NP_758872
DEFINITION = potassium voltage-gated channel, subfamily H, member 1 isoform 1

ACCESSION = NP_004891
DEFINITION = apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B

ACCESSION = NP_115643
DEFINITION = regulator of G-protein signalling like 2

ACCESSION = NP_004643
DEFINITION = ubiquitin specific protease 9, X-linked isoform 1

ACCESSION = XP_951187
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 8

ACCESSION = NP_775659
DEFINITION = phosphodiesterase 8A isoform 4

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_001012276 XP_372762
DEFINITION = PRAME family member 8

ACCESSION = NP_002596
DEFINITION = phosphodiesterase 8A isoform 1

ACCESSION = XP_945571
DEFINITION = PREDICTED: NYD-SP11 protein

ACCESSION = XP_943984
DEFINITION = PREDICTED: similar to SMC hinge domain containing 1

ACCESSION = NP_004516
DEFINITION = low density lipoprotein-related protein 2

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = NP_689639
DEFINITION = apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3D

ACCESSION = NP_003487
DEFINITION = transformation/transcription domain-associated protein

ACCESSION = NP_054750
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_690867
DEFINITION = kelch repeat and BTB (POZ) domain-containing 6

ACCESSION = NP_002702
DEFINITION = protein phosphatase 1 glycogen-binding regulatory subunit 3

ACCESSION = NP_689522
DEFINITION = phosphoinositide-3-kinase adaptor protein 1

ACCESSION = XP_942822
DEFINITION = PREDICTED: similar to TBC1 domain family member 12

ACCESSION = NP_005699
DEFINITION = glypican 6 precursor

ACCESSION = XP_947633
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 2

ACCESSION = NP_001175
DEFINITION = ataxia telangiectasia and Rad3 related protein

ACCESSION = NP_149351
DEFINITION = surfeit 4

ACCESSION = NP_000313
DEFINITION = retinal degeneration slow protein

ACCESSION = XP_371164
DEFINITION = PREDICTED: NYD-SP11 protein

ACCESSION = NP_001439
DEFINITION = glypican 4

ACCESSION = XP_933775

DEFINITION = PREDICTED: similar to CG15133-PA

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = NP_775658
DEFINITION = phosphodiesterase 8A isoform 4

ACCESSION = XP_951179
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 3

ACCESSION = NP_060142
DEFINITION = transient receptor potential cation channel, subfamily M, member 7

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_775656
DEFINITION = phosphodiesterase 8A isoform 2

ACCESSION = NP_004645
DEFINITION = ubiquitin specific protease 9, Y-linked

ACCESSION = NP_001027
DEFINITION = ryanodine receptor 3

ACCESSION = NP_036348
DEFINITION = midline 2 isoform 1

ACCESSION = XP_951188
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 9

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_006819
DEFINITION = activating signal cointegrator 1 complex subunit 3 isoform a

ACCESSION = NP_612409 XP_049384 XP_374590
DEFINITION = nucleolar protein with MIF4G domain 1

ACCESSION = NP_056969
DEFINITION = pre-mRNA cleavage complex II protein Pcf11

ACCESSION = NP_000457
DEFINITION = peroxisome biogenesis factor 1

ACCESSION = NP_001037
DEFINITION = solute carrier family 12 (sodium/potassium/chloride transporters), member 2

ACCESSION = NP_002290
DEFINITION = lactase-phlorizin hydrolase preproprotein

ACCESSION = NP_068706
DEFINITION = ubiquitin specific protease 9, X-linked isoform 2

ACCESSION = NP_055493
DEFINITION = hypothetical protein LOC9701

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = XP_113962
DEFINITION = PREDICTED: structural maintenance of chromosomes flexible hinge domain containing 1

ACCESSION = NP_775657
DEFINITION = phosphodiesterase 8A isoform 3

ACCESSION = NP_055904
DEFINITION = p53-associated parkin-like cytoplasmic protein

ACCESSION = XP_051081
DEFINITION = PREDICTED: TBC1 domain family, member 12

ACCESSION = NP_714544
DEFINITION = stereocilin

ACCESSION = NP_001012277 XP_497651
DEFINITION = PRAME family member 7

ACCESSION = NP_002229
DEFINITION = potassium voltage-gated channel, subfamily H, member 1 isoform 2

ACCESSION = XP_945854

DEFINITION = PREDICTED: similar to Serine-protein kinase ATR (Ataxia telangiectasia and Rad3-related protein) (FRAP-related protein 1)

ACCESSION = NP_065712
DEFINITION = activation-induced cytidine deaminase

ACCESSION = NP_115514
DEFINITION = kelch repeat and BTB (POZ) domain containing 7

ACCESSION = NP_060782
DEFINITION = SAPS domain family, member 3

****
****
The pattern being searched is f.d.f.*DDGLDEAFSRLAQSRT

No matches found

****
****
The pattern being searched is f.d.f.*L[LMIF][DEN][LMIF][DEN]

49 matches found in protein
##########
ACCESSION = NP_060808
DEFINITION = WD repeat domain 52

ACCESSION = NP_079146
DEFINITION = DEP domain containing 2 isoform a

ACCESSION = NP_065965 XP_290768
DEFINITION = chromosome 17 open reading frame 27

ACCESSION = NP_647593
DEFINITION = bridging integrator 1 isoform 1

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_001626
DEFINITION = amphiphysin isoform 1

ACCESSION = NP_058633
DEFINITION = polymerase (DNA-directed), alpha

ACCESSION = XP_947055
DEFINITION = PREDICTED: hypothetical protein LOC57571 isoform 3

ACCESSION = NP_001008536 XP_060104
DEFINITION = trichohyalin-like 1

ACCESSION = NP_055950 XP_371954 XP_374530
DEFINITION = nucleoporin 205kDa

ACCESSION = XP_208522
DEFINITION = PREDICTED: hypothetical protein LOC57571 isoform 1

ACCESSION = NP_085124
DEFINITION = dicer1

ACCESSION = NP_659462
DEFINITION = hypothetical protein LOC221264

ACCESSION = NP_055928
DEFINITION = zinc finger, ZZ type with EF hand domain 1

ACCESSION = NP_060552
DEFINITION = polymerase (RNA) III (DNA directed) polypeptide B

ACCESSION = NP_001617
DEFINITION = v-akt murine thymoma viral oncogene homolog 2

ACCESSION = NP_005839 XP_050909
DEFINITION = c-myc promoter binding protein

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = NP_071373 XP_371779
DEFINITION = transposon-derived Buster3 transposase-like

ACCESSION = NP_003162
DEFINITION = suppressor of var1, 3-like 1

ACCESSION = NP_001355
DEFINITION = chapsyn-110

ACCESSION = NP_937883
DEFINITION = restin isoform b

ACCESSION = NP_005752
DEFINITION = plexin C1

ACCESSION = NP_647477
DEFINITION = amphiphysin isoform 2

ACCESSION = NP_055426
DEFINITION = MDN1, midasin homolog

ACCESSION = NP_001578
DEFINITION = phosphatidylinositol polyphosphate 5-phosphatase isoform b

ACCESSION = NP_002947
DEFINITION = restin isoform a

ACCESSION = NP_055778 XP_375553
DEFINITION = KIAA0963

ACCESSION = NP_001175
DEFINITION = ataxia telangiectasia and Rad3 related protein

ACCESSION = NP_001870
DEFINITION = mannan-binding lectin serine protease 1 isoform 1 precursor

ACCESSION = NP_008983
DEFINITION = NADPH oxidase 1 isoform long

ACCESSION = NP_039249
DEFINITION = NADPH oxidase 1 isoform long variant

ACCESSION = NP_803187
DEFINITION = dicer1

ACCESSION = NP_006486
DEFINITION = ecotropic viral integration site 2B

ACCESSION = NP_000267
DEFINITION = phosphatidylinositol polyphosphate 5-phosphatase isoform a

ACCESSION = NP_039248
DEFINITION = NADPH oxidase 1 isoform short

ACCESSION = XP_942685
DEFINITION = PREDICTED: similar to RIKEN cDNA 4832428D23

ACCESSION = NP_055731
DEFINITION = lemur tyrosine kinase 2

ACCESSION = NP_004800
DEFINITION = stomatin (EPB72)-like 1

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_006819
DEFINITION = activating signal cointegrator 1 complex subunit 3 isoform a

ACCESSION = NP_955452 NP_054844
DEFINITION = DNA polymerase theta

ACCESSION = XP_062872
DEFINITION = PREDICTED: similar to RIKEN cDNA 4832428D23

ACCESSION = NP_079446
DEFINITION = DEP domain containing 2 isoform b

ACCESSION = NP_937762
DEFINITION = laminin alpha 3 subunit isoform 1

ACCESSION = NP_001724
DEFINITION = complement component 1, r subcomponent

ACCESSION = NP_060921
DEFINITION = centromere protein J

ACCESSION = XP_945854
DEFINITION = PREDICTED: similar to Serine-protein kinase ATR (Ataxia telangiectasia and Rad3-related protein) (FRAP-related protein 1)

ACCESSION = NP_001814
DEFINITION = brain creatine kinase

****
****
The pattern being searched is f.d.f.*npf

52 matches found in protein
##############################
ACCESSION = NP_001017372
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_005622
DEFINITION = smoothened

ACCESSION = NP_757343
DEFINITION = calcium/calmodulin-dependent protein kinase 1 alpha isoform a

ACCESSION = NP_065997 XP_497076
DEFINITION = hypothetical protein LOC57706 isoform 1

ACCESSION = NP_115643
DEFINITION = regulator of G-protein signalling like 2

ACCESSION = NP_004643
DEFINITION = ubiquitin specific protease 9, X-linked isoform 1

ACCESSION = NP_004341
DEFINITION = runt-related transcription factor 3 isoform 2

ACCESSION = NP_000042
DEFINITION = ataxia telangiectasia mutated protein isoform 1

ACCESSION = NP_002412
DEFINITION = matrix metalloproteinase 1 preproprotein

ACCESSION = NP_757344
DEFINITION = calcium/calmodulin-dependent protein kinase 1 alpha isoform b

ACCESSION = NP_652761
DEFINITION = A-kinase anchor protein 11 isoform 2

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_056372
DEFINITION = chromodomain helicase DNA binding protein 5

ACCESSION = XP_943674
DEFINITION = PREDICTED: similar to pleckstrin homology domain containing, family M (with RUN domain) member 1

ACCESSION = NP_036586
DEFINITION = T-cell lymphoma invasion and metastasis 2 isoform a

ACCESSION = NP_001226 NP_004344
DEFINITION = serine (or cysteine) proteinase inhibitor, clade H, member 1 precursor

ACCESSION = NP_005860
DEFINITION = serologically defined colon cancer antigen 10

ACCESSION = NP_001018047
DEFINITION = vacuolar protein sorting 13A isoform C

ACCESSION = NP_054750
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_056001
DEFINITION = vacuolar protein sorting 13A isoform B

ACCESSION = NP_006785
DEFINITION = G protein-coupled receptor 75

ACCESSION = NP_001026850
DEFINITION = runt-related transcription factor 3 isoform 1

ACCESSION = NP_000523
DEFINITION = propionyl Coenzyme A carboxylase, beta polypeptide

ACCESSION = NP_001438
DEFINITION = FAT tumor suppressor 2 precursor

ACCESSION = NP_002539
DEFINITION = olfactory receptor, family 1, subfamily D, member 2

ACCESSION = NP_005586
DEFINITION = nuclear factor I/A

ACCESSION = NP_056090 XP_035313
DEFINITION = hypothetical protein LOC23325

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = XP_944522
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_002967
DEFINITION = sodium channel, voltage-gated, type VII, alpha

ACCESSION = NP_060142
DEFINITION = transient receptor potential cation channel, subfamily M, member 7

ACCESSION = NP_001101
DEFINITION = ADAM metallopeptidase domain 10

ACCESSION = NP_003256
DEFINITION = toll-like receptor 3

ACCESSION = NP_004645
DEFINITION = ubiquitin specific protease 9, Y-linked

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_071881
DEFINITION = sterolin 1

ACCESSION = NP_001018048
DEFINITION = vacuolar protein sorting 13A isoform D

ACCESSION = NP_055921
DEFINITION = RAD54-like 2

ACCESSION = NP_001017970 XP_090844
DEFINITION = transmembrane protein 30B

ACCESSION = NP_612409 XP_049384 XP_374590
DEFINITION = nucleolar protein with MIF4G domain 1

ACCESSION = NP_150648
DEFINITION = vacuolar protein sorting 13A isoform A

ACCESSION = NP_955452 NP_054844
DEFINITION = DNA polymerase theta

ACCESSION = NP_000341
DEFINITION = ATP-binding cassette, sub-family A member 4

ACCESSION = NP_057332
DEFINITION = A-kinase anchor protein 11 isoform 1

ACCESSION = NP_068706
DEFINITION = ubiquitin specific protease 9, X-linked isoform 2

ACCESSION = NP_060676
DEFINITION = vacuolar protein sorting 35

ACCESSION = NP_056028 XP_290550
DEFINITION = RAB6 interacting protein 1

ACCESSION = XP_290944
DEFINITION = PREDICTED: pleckstrin homology domain containing, family M (with RUN domain) member 2

ACCESSION = NP_115670
DEFINITION = calcium/calmodulin-dependent protein kinase 1 alpha isoform a

ACCESSION = NP_003543 XP_375390
DEFINITION = olfactory receptor, family 1, subfamily D, member 4

ACCESSION = XP_945884
DEFINITION = PREDICTED: similar to Serine-protein kinase ATM (Ataxia telangiectasia mutated) (A-T, mutated)

ACCESSION = NP_057665
DEFINITION = evolutionarily conserved signaling intermediate in Toll pathway

****
****
The pattern being searched is W..[FW].[DE].*dp[fw]

66 matches found in protein
############################
ACCESSION = XP_055481
DEFINITION = PREDICTED: myb-like, SWIRM and MPN domains 1

ACCESSION = NP_006142 XP_042207
DEFINITION = lactoperoxidase

ACCESSION = XP_945719
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 1

ACCESSION = NP_055680
DEFINITION = chromosome condensation-related SMC-associated protein 1

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_543148
DEFINITION = netrin receptor Unc5h4

ACCESSION = XP_941069
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 8

ACCESSION = NP_065816
DEFINITION = retinoblastoma-associated factor 600

ACCESSION = NP_573570
DEFINITION = PGC-1-related estrogen receptor alpha coactivator

ACCESSION = XP_946889
DEFINITION = PREDICTED: similar to myb-like, SWIRM and MPN domains 1

ACCESSION = XP_935639
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 1

ACCESSION = NP_001026971
DEFINITION = LIM domain kinase 2 isoform 1

ACCESSION = NP_056076 XP_166201
DEFINITION = KIAA0056 protein

ACCESSION = XP_373030
DEFINITION = PREDICTED: hypothetical protein XP_373030

ACCESSION = NP_005560
DEFINITION = LIM domain kinase 2 isoform 2a

ACCESSION = XP_940464
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 3

ACCESSION = NP_055726
DEFINITION = AP2 associated kinase 1

ACCESSION = NP_061888 XP_376724 XP_379974
DEFINITION = chondroitin sulfate glucuronyltransferase

ACCESSION = NP_004545
DEFINITION = cytoplasmic nuclear factor of activated T-cells 4

ACCESSION = NP_942595
DEFINITION = BMP-2 inducible kinase isoform a

ACCESSION = NP_054860
DEFINITION = cell recognition molecule Caspr2 precursor

ACCESSION = NP_005916
DEFINITION = meprin A, beta

ACCESSION = XP_933562
DEFINITION = PREDICTED: hypothetical protein XP_933562

ACCESSION = XP_940790
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 3

ACCESSION = NP_064569
DEFINITION = cyclin M4

ACCESSION = NP_115971 XP_496236
DEFINITION = ubiquitin specific protease 32

ACCESSION = XP_949797
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 6

ACCESSION = NP_056534
DEFINITION = collagen, type V, alpha 3 preproprotein

ACCESSION = NP_689914
DEFINITION = ATP binding cassette, sub-family A (ABC1), member 13

ACCESSION = NP_690867
DEFINITION = kelch repeat and BTB (POZ) domain-containing 6

ACCESSION = NP_697043
DEFINITION = retinoblastoma binding protein 9

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = NP_689522
DEFINITION = phosphoinositide-3-kinase adaptor protein 1

ACCESSION = NP_002410
DEFINITION = mitogen-activated protein kinase kinase kinase 11

ACCESSION = NP_113584 NP_005694 NP_060097 XP_497119
DEFINITION = HECT, UBA and WWE domain containing 1

ACCESSION = XP_949782
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 3

ACCESSION = NP_060753
DEFINITION = nudix-type motif 15

ACCESSION = NP_001395
DEFINITION = eukaryotic translation elongation factor 1 gamma

ACCESSION = NP_004066
DEFINITION = cryptochrome 1 (photolyase-like)

ACCESSION = NP_689494
DEFINITION = NTKL-binding protein 1

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_114128
DEFINITION = RALBP1 associated Eps domain containing 1

ACCESSION = NP_065870 XP_371832
DEFINITION = hypothetical protein LOC57579

ACCESSION = NP_000183
DEFINITION = T-box 5 isoform 1

ACCESSION = XP_940468
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 6

ACCESSION = NP_061897 XP_042685
DEFINITION = hypothetical protein LOC54497

ACCESSION = XP_933588
DEFINITION = PREDICTED: similar to vasoactive intestinal peptide receptor 2

ACCESSION = NP_006597
DEFINITION = retinoblastoma binding protein 9

ACCESSION = NP_741996
DEFINITION = sal-like 3

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = XP_941064
DEFINITION = PREDICTED: similar to Elongation factor 1-gamma (EF-1-gamma) (eEF-1B gamma) isoform 5

ACCESSION = NP_542448

DEFINITION = T-box 5 isoform 3

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_542449
DEFINITION = T-box 5 isoform 2

ACCESSION = NP_002323
DEFINITION = low density lipoprotein-related protein 1

ACCESSION = NP_060958
DEFINITION = T-box 4

ACCESSION = NP_060414
DEFINITION = ubiquitin specific protease 47

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_060225
DEFINITION = NOL1/NOP2/Sun domain family 2 protein

ACCESSION = NP_005246
DEFINITION = cyclin G associated kinase

ACCESSION = NP_057952
DEFINITION = LIM domain kinase 2 isoform 2b

ACCESSION = NP_061027
DEFINITION = low density lipoprotein-related protein 1B

ACCESSION = NP_066267
DEFINITION = ankyrin 3 isoform 1

ACCESSION = XP_944598
DEFINITION = PREDICTED: hypothetical protein XP_944598

ACCESSION = NP_115514
DEFINITION = kelch repeat and BTB (POZ) domain containing 7

ACCESSION = NP_852259
DEFINITION = T-box 5 isoform 1

****
****
The pattern being searched is W..[FW].[DE].*f.d.f

54 matches found in protein
##########
ACCESSION = NP_004232 XP_495826
DEFINITION = jumonji domain containing 1C

ACCESSION = NP_005466
DEFINITION = lymphocyte adaptor protein

ACCESSION = NP_003997
DEFINITION = dystrophin Dp427m isoform

ACCESSION = XP_947086
DEFINITION = PREDICTED: similar to stereocilin

ACCESSION = NP_065816
DEFINITION = retinoblastoma-associated factor 600

ACCESSION = NP_055993
DEFINITION = Rho-related BTB domain containing 2

ACCESSION = NP_597812
DEFINITION = sodium bicarbonate transporter 4 isoform c

ACCESSION = NP_055595
DEFINITION = cullin 7

ACCESSION = NP_060009
DEFINITION = dynein, axonemal, heavy polypeptide 3

ACCESSION = NP_001260
DEFINITION = regulator of chromosome condensation 1

ACCESSION = NP_002213
DEFINITION = inositol 1,4,5-triphosphate receptor, type 1

ACCESSION = NP_001377
DEFINITION = dihydropyrimidinase-like 2

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_055869 XP_045423
DEFINITION = hypothetical protein LOC23074 isoform a

ACCESSION = NP_477513
DEFINITION = monoacylglycerol O-acyltransferase 1

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = NP_067019
DEFINITION = sodium bicarbonate transporter 4 isoform a

ACCESSION = NP_066189
DEFINITION = adaptor protein with pleckstrin homology and src homology 2 domains

ACCESSION = NP_006606
DEFINITION = calpain 9 isoform 1

ACCESSION = NP_631905
DEFINITION = striated muscle activator of Rho-dependent signaling

ACCESSION = NP_055651
DEFINITION = Rho-related BTB domain containing 1

ACCESSION = NP_597813
DEFINITION = sodium bicarbonate transporter 4 isoform d

ACCESSION = NP_001027552
DEFINITION = Rho-related BTB domain containing 1

ACCESSION = XP_947633
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 2

ACCESSION = NP_055051
DEFINITION = glyceronephosphate O-acyltransferase

ACCESSION = NP_004001
DEFINITION = dystrophin Dp427p2 isoform

ACCESSION = NP_001026849
DEFINITION = methionine sulfoxide reductase B3 isoform 2

ACCESSION = NP_000100
DEFINITION = dystrophin Dp427c isoform

ACCESSION = NP_056466 XP_045308
DEFINITION = PHD finger protein 19 isoform a

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = NP_004000
DEFINITION = dystrophin Dp427p1 isoform

ACCESSION = NP_001032
DEFINITION = sucrase-isomaltase (alpha-glucosidase)

ACCESSION = XP_951179
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 3

ACCESSION = NP_115536
DEFINITION = hypothetical protein LOC92126

ACCESSION = NP_057688
DEFINITION = jumonji domain containing 1B

ACCESSION = NP_006589
DEFINITION = solute carrier family 12 (potassium/chloride transporters), member 7

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_061720
DEFINITION = axonemal dynein heavy chain 7

ACCESSION = NP_201580
DEFINITION = sodium bicarbonate transporter 4 isoform b

ACCESSION = XP_951188

DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 9

ACCESSION = NP_071341
DEFINITION = solute carrier family 4, sodium bicarbonate transporter-like, member 10

ACCESSION = NP_612152
DEFINITION = polycystin-1L1

ACCESSION = NP_002290
DEFINITION = lactase-phlorizin hydrolase preproprotein

ACCESSION = NP_055493
DEFINITION = hypothetical protein LOC9701

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_060903
DEFINITION = jumonji domain containing 1A

ACCESSION = NP_055904
DEFINITION = p53-associated parkin-like cytoplasmic protein

ACCESSION = NP_937868
DEFINITION = Rho-related BTB domain containing 1

ACCESSION = NP_055178
DEFINITION = sacsin

ACCESSION = NP_714544
DEFINITION = stereocilin

ACCESSION = NP_003989
DEFINITION = nuclear factor kappa-B, subunit 1

ACCESSION = NP_932346
DEFINITION = methionine sulfoxide reductase B3 isoform 1

ACCESSION = NP_003998
DEFINITION = dystrophin Dp427l isoform

ACCESSION = NP_031384
DEFINITION = metal response element-binding transcription factor 2

****
****
The pattern being searched is W..[FW].[DE].*W..[FW].[DE]

98 matches found in protein
##########
ACCESSION = NP_004232 XP_495826
DEFINITION = jumonji domain containing 1C

ACCESSION = NP_079021
DEFINITION = SHC SH2-domain binding protein 1

ACCESSION = XP_940910
DEFINITION = PREDICTED: similar to radical S-adenosyl methionine and flavodoxin domains 1 isoform 6

ACCESSION = XP_940913
DEFINITION = PREDICTED: similar to radical S-adenosyl methionine and flavodoxin domains 1 isoform 7

ACCESSION = NP_543148
DEFINITION = netrin receptor Unc5h4

ACCESSION = NP_006286
DEFINITION = valyl-tRNA synthetase

ACCESSION = NP_149062
DEFINITION = nesprin 1 isoform longer

ACCESSION = NP_079406
DEFINITION = hexokinase domain containing 1

ACCESSION = NP_055595
DEFINITION = cullin 7

ACCESSION = XP_942900
DEFINITION = PREDICTED: similar to dynein, cytoplasmic, heavy chain 2 isoform 3

ACCESSION = NP_056327
DEFINITION = dynein, axonemal, heavy polypeptide 1

ACCESSION = NP_001363
DEFINITION = dynein, axonemal, heavy polypeptide 9 isoform 2

ACCESSION = NP_842565
DEFINITION = spectrin, beta, non-erythrocytic 1 isoform 2

ACCESSION = XP_937109
DEFINITION = PREDICTED: hypothetical protein LOC441250 isoform 3

ACCESSION = NP_277033
DEFINITION = hexokinase 1 isoform HKI-ta/tb

ACCESSION = NP_065779
DEFINITION = family with sequence similarity 62 (C2 domain containing) member B

ACCESSION = NP_878918
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform e

ACCESSION = NP_149992 XP_372210
DEFINITION = protein phosphatase 1, regulatory (inhibitor) subunit 3F

ACCESSION = NP_006030
DEFINITION = mannose receptor, C type 2

ACCESSION = NP_064616
DEFINITION = hypothetical protein LOC56983

ACCESSION = NP_003768
DEFINITION = dynein, axonemal, heavy polypeptide 11

ACCESSION = XP_950850
DEFINITION = PREDICTED: similar to subcommissural organ spondin isoform 16

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_056108
DEFINITION = nesprin 1 isoform beta

ACCESSION = NP_060127
DEFINITION = aftiphilin protein isoform b

ACCESSION = XP_942394
DEFINITION = PREDICTED: similar to CG7400-PA, isoform A isoform 2

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = NP_000329
DEFINITION = sodium potassium chloride cotransporter 2

ACCESSION = NP_277031
DEFINITION = hexokinase 1 isoform HKI-R

ACCESSION = NP_277035
DEFINITION = hexokinase 1 isoform HKI-td

ACCESSION = NP_003322
DEFINITION = tyrosine kinase 2

ACCESSION = XP_933562
DEFINITION = PREDICTED: hypothetical protein XP_933562

ACCESSION = NP_612815
DEFINITION = BCL2-like 1 isoform 1

ACCESSION = NP_689518
DEFINITION = hypothetical protein LOC56983

ACCESSION = NP_689914
DEFINITION = ATP binding cassette, sub-family A (ABC1), member 13

ACCESSION = NP_003096
DEFINITION = sortilin-related receptor containing LDLR class A repeats preproprotein

ACCESSION = NP_001007468 XP_038520
DEFINITION = spindle assembly associated Sfi1 homolog isoform a

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = NP_001367
DEFINITION = dynein, cytoplasmic, heavy polypeptide 1

ACCESSION = NP_055433
DEFINITION = deleted in bladder cancer 1

ACCESSION = NP_062548
DEFINITION = butyrophilin-like 2

ACCESSION = NP_060734
DEFINITION = radical S-adenosyl methionine and flavodoxin domains 1

ACCESSION = XP_947633
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 2

ACCESSION = NP_001362
DEFINITION = dynein, axonemal, heavy polypeptide 8

ACCESSION = NP_149095
DEFINITION = stonin 2

ACCESSION = NP_000180
DEFINITION = hexokinase 2

ACCESSION = NP_982261
DEFINITION = aftiphilin protein isoform a

ACCESSION = XP_940855
DEFINITION = PREDICTED: similar to subcommissural organ spondin isoform 7

ACCESSION = NP_000118
DEFINITION = exostosin 1

ACCESSION = NP_055051
DEFINITION = glyceronephosphate O-acyltransferase

ACCESSION = NP_872372
DEFINITION = secretory protein LOC284013

ACCESSION = XP_371164
DEFINITION = PREDICTED: NYD-SP11 protein

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = XP_937099
DEFINITION = PREDICTED: hypothetical protein LOC441250 isoform 2

ACCESSION = NP_001032
DEFINITION = sucrase-isomaltase (alpha-glucosidase)

ACCESSION = XP_951179
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 3

ACCESSION = NP_002325 XP_035037
DEFINITION = low density lipoprotein receptor-related protein 4

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_001945
DEFINITION = discoidin domain receptor family, member 1 isoform b

ACCESSION = NP_114128
DEFINITION = RALBP1 associated Eps domain containing 1

ACCESSION = NP_005145
DEFINITION = ubiquitin specific protease 8

ACCESSION = XP_039877
DEFINITION = PREDICTED: mucin 5, subtype B, tracheobronchial

ACCESSION = NP_057736
DEFINITION = crooked neck-like 1 protein

ACCESSION = NP_002327
DEFINITION = low density lipoprotein receptor-related protein 6

ACCESSION = NP_006099 NP_116109
DEFINITION = spondin 1, extracellular matrix protein

ACCESSION = NP_071375
DEFINITION = CTF18, chromosome transmission fidelity factor 18 homolog

ACCESSION = NP_001360
DEFINITION = dynein, axonemal, heavy polypeptide 5

ACCESSION = XP_951188
DEFINITION = PREDICTED: similar to Protein KIAA0685 isoform 9

ACCESSION = NP_071341
DEFINITION = solute carrier family 4, sodium bicarbonate transporter-like, member 10

ACCESSION = XP_933588
DEFINITION = PREDICTED: similar to vasoactive intestinal peptide receptor 2

ACCESSION = NP_000179
DEFINITION = hexokinase 1 isoform HKI

ACCESSION = NP_001002243
DEFINITION = aftiphilin protein isoform c

ACCESSION = XP_943930
DEFINITION = PREDICTED: similar to Mucin-5B precursor (Mucin 5 subtype B, tracheobronchial) (High molecular weight salivary mucin MG1) (Sublingual gland mucin)

ACCESSION = NP_002326
DEFINITION = low density lipoprotein receptor-related protein 5

ACCESSION = NP_776297
DEFINITION = chloride channel 3 isoform e

ACCESSION = XP_937111
DEFINITION = PREDICTED: hypothetical protein LOC441250 isoform 4

ACCESSION = XP_940915
DEFINITION = PREDICTED: similar to radical S-adenosyl methionine and flavodoxin domains 1 isoform 8

ACCESSION = XP_371850
DEFINITION = PREDICTED: similar to CaLPain family member (clp-2)

ACCESSION = NP_002323
DEFINITION = low density lipoprotein-related protein 1

ACCESSION = XP_940626
DEFINITION = PREDICTED: similar to radical S-adenosyl methionine and flavodoxin domains 1 isoform 5

ACCESSION = NP_002290
DEFINITION = lactase-phlorizin hydrolase preproprotein

ACCESSION = NP_055907 NP_054725
DEFINITION = PI-3-kinase-related kinase SMG-1

ACCESSION = NP_892006
DEFINITION = nesprin 1 longest

ACCESSION = NP_000780
DEFINITION = angiotensin I converting enzyme isoform 1 precursor

ACCESSION = NP_060414
DEFINITION = ubiquitin specific protease 47

ACCESSION = NP_055493
DEFINITION = hypothetical protein LOC9701

ACCESSION = NP_056483
DEFINITION = regulator of G-protein signalling 22

ACCESSION = NP_060903
DEFINITION = jumonji domain containing 1A

ACCESSION = XP_370652
DEFINITION = PREDICTED: dynein, cytoplasmic, heavy polypeptide 2 isoform 1

ACCESSION = NP_055590 XP_038520
DEFINITION = spindle assembly associated Sfi1 homolog isoform b

ACCESSION = NP_001820
DEFINITION = chloride channel 3 isoform b

ACCESSION = XP_946413
DEFINITION = PREDICTED: similar to CaLPain family member (clp-2)

ACCESSION = NP_061027
DEFINITION = low density lipoprotein-related protein 1B

ACCESSION = XP_932438

DEFINITION = PREDICTED: hypothetical protein LOC441250 isoform 1

ACCESSION = NP_277032
DEFINITION = hexokinase 1 isoform HKI-ta/tb

ACCESSION = NP_003119
DEFINITION = spectrin, beta, non-erythrocytic 1 isoform 1

ACCESSION = NP_004446
DEFINITION = exostoses (multiple)-like 1

ACCESSION = NP_003461
DEFINITION = ubiquitin specific protease 7 (herpes virus-associated)

****
****
The pattern being searched is W..[FW].[DE].*DDGLDEAFSRLAQSRT

No matches found

****
****
The pattern being searched is W..[FW].[DE].*L[LMIF][DEN][LMIF][DEN]

47 matches found in protein
##########
ACCESSION = NP_078946
DEFINITION = suppressor of variegation 3-9 homolog 2

ACCESSION = NP_149062
DEFINITION = nesprin 1 isoform longer

ACCESSION = NP_597812
DEFINITION = sodium bicarbonate transporter 4 isoform c

ACCESSION = NP_060392
DEFINITION = PRP39 pre-mRNA processing factor 39 homolog

ACCESSION = NP_001363
DEFINITION = dynein, axonemal, heavy polypeptide 9 isoform 2

ACCESSION = NP_001026971
DEFINITION = LIM domain kinase 2 isoform 1

ACCESSION = NP_065779
DEFINITION = family with sequence similarity 62 (C2 domain containing) member B

ACCESSION = NP_877435 XP_170760
DEFINITION = hypothetical protein LOC256764

ACCESSION = XP_043739
DEFINITION = PREDICTED: dual specificity phosphatase 27 (putative) isoform 1

ACCESSION = NP_937879
DEFINITION = Rho guanine nucleotide exchange factor (GEF) 11 isoform 2

ACCESSION = NP_002232
DEFINITION = potassium inwardly-rectifying channel J10

ACCESSION = NP_003772
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = NP_788276
DEFINITION = CDK5 regulatory subunit associated protein 3 isoform b

ACCESSION = NP_067019
DEFINITION = sodium bicarbonate transporter 4 isoform a

ACCESSION = NP_000553
DEFINITION = complement component 8, alpha polypeptide precursor

ACCESSION = NP_056107
DEFINITION = family with sequence similarity 62 (C2 domain containing), member A

ACCESSION = NP_003031
DEFINITION = solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1)

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = XP_947521
DEFINITION = PREDICTED: similar to Dual specificity protein phosphatase 13 (Testis- and skeletal-muscle-specific DSP) isoform 4

ACCESSION = NP_057123
DEFINITION = chromosome 14 open reading frame 166

ACCESSION = NP_597813
DEFINITION = sodium bicarbonate transporter 4 isoform d

ACCESSION = NP_963868
DEFINITION = solute carrier family 4, anion exchanger, member 3 isoform 2

ACCESSION = NP_149358
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_001362
DEFINITION = dynein, axonemal, heavy polypeptide 8

ACCESSION = NP_982261
DEFINITION = aftiphilin protein isoform a

ACCESSION = NP_057733
DEFINITION = ABT1-associated protein

ACCESSION = NP_149359
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_055599
DEFINITION = Rho guanine nucleotide exchange factor (GEF) 11 isoform 1

ACCESSION = NP_004644
DEFINITION = Smcy homolog, Y-linked

ACCESSION = NP_001175
DEFINITION = ataxia telangiectasia and Rad3 related protein

ACCESSION = NP_006210
DEFINITION = phosphoinositide-3-kinase, catalytic, beta polypeptide

ACCESSION = NP_004178
DEFINITION = Smcx homolog, X chromosome

ACCESSION = NP_149115
DEFINITION = hypothetical protein LOC85478

ACCESSION = NP_114128
DEFINITION = RALBP1 associated Eps domain containing 1

ACCESSION = NP_201580
DEFINITION = sodium bicarbonate transporter 4 isoform b

ACCESSION = NP_006835
DEFINITION = ilvB (bacterial acetolactate synthase)-like isoform 1

ACCESSION = NP_005061
DEFINITION = solute carrier family 4, anion exchanger, member 3 isoform 1

ACCESSION = NP_006819
DEFINITION = activating signal cointegrator 1 complex subunit 3 isoform a

ACCESSION = NP_699187
DEFINITION = TBC1 domain family, member 21

ACCESSION = NP_149357
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_892006
DEFINITION = nesprin 1 longest

ACCESSION = NP_000780
DEFINITION = angiotensin I converting enzyme isoform 1 precursor

ACCESSION = NP_777576
DEFINITION = ubiquitin protein ligase E3 component n-recognin 1

ACCESSION = NP_004228
DEFINITION = thyroid hormone receptor interactor 13

ACCESSION = XP_945854
DEFINITION = PREDICTED: similar to Serine-protein kinase ATR (Ataxia telangiectasia and Rad3-related protein) (FRAP-related protein 1)

ACCESSION = NP_061159 XP_051860
DEFINITION = KIAA1199

****
****
The pattern being searched is W..[FW].[DE].*npf

62 matches found in protein
##############################
ACCESSION = NP_058650
DEFINITION = T-box 22

ACCESSION = XP_943361
DEFINITION = PREDICTED: similar to T-box transcription factor TBX18 (T-box protein 18) isoform 2

ACCESSION = NP_001017372
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_065855
DEFINITION = protein kinase C and casein kinase substrate in neurons 1

ACCESSION = NP_056120
DEFINITION = angel homolog 1

ACCESSION = XP_942900
DEFINITION = PREDICTED: similar to dynein, cytoplasmic, heavy chain 2 isoform 3

ACCESSION = NP_689593 XP_371284
DEFINITION = T-box 15

ACCESSION = NP_115643
DEFINITION = regulator of G-protein signalling like 2

ACCESSION = NP_004643
DEFINITION = ubiquitin specific protease 9, X-linked isoform 1

ACCESSION = NP_065872
DEFINITION = vacuolar protein sorting 13C protein isoform 2A

ACCESSION = NP_001018098
DEFINITION = vacuolar protein sorting 13C protein isoform 2B

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_003990
DEFINITION = oncostatin M receptor

ACCESSION = NP_003585
DEFINITION = transcription termination factor, RNA polymerase II

ACCESSION = NP_006436
DEFINITION = U5 snRNP-specific protein

ACCESSION = NP_003772
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_009160
DEFINITION = protein kinase C and casein kinase substrate in neurons 2

ACCESSION = NP_114119 XP_291067
DEFINITION = family with sequence similarity 62 (C2 domain containing), member C

ACCESSION = NP_631905
DEFINITION = striated muscle activator of Rho-dependent signaling

ACCESSION = NP_054750
DEFINITION = solute carrier family 27 (fatty acid transporter), member 6

ACCESSION = NP_005140
DEFINITION = T-box 19

ACCESSION = NP_079170
DEFINITION = nucleolar protein 10

ACCESSION = NP_055433
DEFINITION = deleted in bladder cancer 1

ACCESSION = NP_002410
DEFINITION = mitogen-activated protein kinase kinase kinase 11

ACCESSION = NP_149358
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_149095
DEFINITION = stonin 2

ACCESSION = NP_149359
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_060550
DEFINITION = vacuolar protein sorting 13C protein isoform 1B

ACCESSION = NP_036546
DEFINITION = rab3 GTPase-activating protein, non-catalytic subunit

ACCESSION = NP_055797
DEFINITION = pecanex homolog

ACCESSION = NP_115597
DEFINITION = chromodomain helicase DNA binding protein 6

ACCESSION = NP_036578
DEFINITION = single-stranded DNA binding protein 2

ACCESSION = NP_057323
DEFINITION = myosin XV

ACCESSION = NP_001032
DEFINITION = sucrase-isomaltase (alpha-glucosidase)

ACCESSION = NP_060142
DEFINITION = transient receptor potential cation channel, subfamily M, member 7

ACCESSION = NP_003172
DEFINITION = transcription factor T

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_078922 XP_038291
DEFINITION = hypothetical protein LOC79699

ACCESSION = NP_004645
DEFINITION = ubiquitin specific protease 9, Y-linked

ACCESSION = NP_000183
DEFINITION = T-box 5 isoform 1

ACCESSION = NP_065150
DEFINITION = T-box transcription factor TBX20

ACCESSION = NP_001009944
DEFINITION = polycystin 1 isoform 1 precursor

ACCESSION = NP_542448
DEFINITION = T-box 5 isoform 3

ACCESSION = NP_776297
DEFINITION = chloride channel 3 isoform e

ACCESSION = NP_060154
DEFINITION = vacuolar protein sorting 13C protein isoform 1A

ACCESSION = XP_496819
DEFINITION = PREDICTED: T-box 18 isoform 1

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_001821
DEFINITION = chloride channel 4

ACCESSION = NP_542449
DEFINITION = T-box 5 isoform 2

ACCESSION = NP_060958
DEFINITION = T-box 4

ACCESSION = NP_149357
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_060250 XP_098762
DEFINITION = chromodomain helicase DNA binding protein 7

ACCESSION = NP_068706
DEFINITION = ubiquitin specific protease 9, X-linked isoform 2

ACCESSION = NP_000287
DEFINITION = polycystin 1 isoform 2 precursor

ACCESSION = XP_370652
DEFINITION = PREDICTED: dynein, cytoplasmic, heavy polypeptide 2 isoform 1

ACCESSION = NP_060225
DEFINITION = NOL1/NOP2/Sun domain family 2 protein

ACCESSION = NP_001820
DEFINITION = chloride channel 3 isoform b

ACCESSION = NP_066267
DEFINITION = ankyrin 3 isoform 1

ACCESSION = NP_055524 XP_291018
DEFINITION = ubiquitin specific protease 34

ACCESSION = NP_061903
DEFINITION = DEAH (Asp-Glu-Ala-His) box polypeptide 29

ACCESSION = NP_852259
DEFINITION = T-box 5 isoform 1

ACCESSION = NP_001262
DEFINITION = chromodomain helicase DNA binding protein 2

****
****
The pattern being searched is DDGLDEAFSRLAQSRT.*dp[fw]

No matches found

****
****
The pattern being searched is DDGLDEAFSRLAQSRT.*f.d.f

No matches found

****
****
The pattern being searched is DDGLDEAFSRLAQSRT.*W..[FW].[DE]

No matches found

****
****
The pattern being searched is DDGLDEAFSRLAQSRT.*DDGLDEAFSRLAQSRT

No matches found

****
****
The pattern being searched is DDGLDEAFSRLAQSRT.*L[LMIF][DEN][LMIF][DEN]

No matches found

****
****
The pattern being searched is DDGLDEAFSRLAQSRT.*npf

No matches found

****
****
The pattern being searched is L[LMIF][DEN][LMIF][DEN].*dp[fw]

85 matches found in protein
##############################
ACCESSION = NP_079146
DEFINITION = DEP domain containing 2 isoform a

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_057318
DEFINITION = acyl-CoA synthetase long-chain family member 5 isoform a

ACCESSION = NP_001626
DEFINITION = amphiphysin isoform 1

ACCESSION = NP_057368
DEFINITION = CCR4-NOT transcription complex, subunit 1 isoform a

ACCESSION = NP_001028927
DEFINITION = copine-like protein

ACCESSION = NP_065916
DEFINITION = DEAH (Asp-Glu-Ala-His) box polypeptide 36

ACCESSION = XP_945898
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 12

ACCESSION = NP_110403
DEFINITION = tumor stroma and activated macrophage protein DLM-1

ACCESSION = NP_976313
DEFINITION = acyl-CoA synthetase long-chain family member 5 isoform b

ACCESSION = NP_071451
DEFINITION = melanoma differentiation associated protein-5

ACCESSION = NP_071407
DEFINITION = cadherin related 23 isoform 1 precursor

ACCESSION = XP_941081
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 11

ACCESSION = XP_941078
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 8

ACCESSION = NP_060653
DEFINITION = sno, strawberry notch homolog 1

ACCESSION = NP_005329
DEFINITION = huntingtin interacting protein 1

ACCESSION = NP_001008536 XP_060104
DEFINITION = trichohyalin-like 1

ACCESSION = XP_949896
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 14

ACCESSION = NP_085124
DEFINITION = dicer1

ACCESSION = XP_942343
DEFINITION = PREDICTED: similar to purinergic receptor P2X3

ACCESSION = NP_149045
DEFINITION = protocadherin 15 precursor

ACCESSION = XP_946300
DEFINITION = PREDICTED: similar to FRAS1-related extracellular matrix protein 2 precursor (ECM3 homolog)

ACCESSION = XP_934977
DEFINITION = PREDICTED: FRAS1 related extracellular matrix protein 2

ACCESSION = XP_941079
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 9

ACCESSION = NP_001005336
DEFINITION = dynamin 1 isoform 2

ACCESSION = NP_443715
DEFINITION = WD repeat domain 10 isoform 2

ACCESSION = XP_943674
DEFINITION = PREDICTED: similar to pleckstrin homology domain containing, family M (with RUN domain) member 1

ACCESSION = NP_065901 XP_043069
DEFINITION = RAN binding protein 10

ACCESSION = XP_939215
DEFINITION = PREDICTED: piccolo isoform 6

ACCESSION = NP_004406

DEFINITION = desmoplakin isoform I

ACCESSION = NP_003889
DEFINITION = synaptojanin 2

ACCESSION = NP_003725
DEFINITION = amine oxidase, copper containing 3 precursor

ACCESSION = NP_776152
DEFINITION = PDZ domain containing 8

ACCESSION = NP_689914
DEFINITION = ATP binding cassette, sub-family A (ABC1), member 13

ACCESSION = NP_002550
DEFINITION = purinergic receptor P2X3

ACCESSION = NP_149132 XP_027237
DEFINITION = mitogen-activated protein kinase kinase kinase 9

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = NP_877439 XP_294213 XP_353565
DEFINITION = putative binding protein 7a5

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = XP_949903
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 16

ACCESSION = NP_004942
DEFINITION = EBV-induced G protein-coupled receptor 2

ACCESSION = NP_758424
DEFINITION = ATP-binding cassette, sub-family A , member 5

ACCESSION = NP_647477
DEFINITION = amphiphysin isoform 2

ACCESSION = NP_060779
DEFINITION = hypothetical protein LOC55773

ACCESSION = NP_009097
DEFINITION = phosphatidylinositol-binding clathrin assembly protein isoform 1

ACCESSION = NP_003728
DEFINITION = dachsous 1 precursor

ACCESSION = XP_939207
DEFINITION = PREDICTED: piccolo isoform 4

ACCESSION = NP_525023
DEFINITION = ATP-binding cassette, sub-family A, member 6

ACCESSION = NP_055968
DEFINITION = PHD finger protein 3

ACCESSION = XP_942238
DEFINITION = PREDICTED: similar to myoferlin isoform b

ACCESSION = NP_055811 XP_042635
DEFINITION = phospholipase C-like 3

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = NP_149072
DEFINITION = death inducer-obliterator 1 isoform c

ACCESSION = NP_002709
DEFINITION = protein phosphatase 2, regulatory subunit B", alpha isoform 1

ACCESSION = XP_949889
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 13

ACCESSION = NP_003924
DEFINITION = BAI1-associated protein 3

ACCESSION = NP_803187
DEFINITION = dicer1

ACCESSION = NP_055434
DEFINITION = glutamate receptor KA1 precursor

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_065870 XP_371832
DEFINITION = hypothetical protein LOC57579

ACCESSION = NP_001010855 XP_375404
DEFINITION = hypothetical protein LOC146850

ACCESSION = NP_055656 XP_498116
DEFINITION = synaptosomal-associated protein, 91kDa homolog

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_055919 XP_290517
DEFINITION = hypothetical protein LOC23130

ACCESSION = NP_078858
DEFINITION = FAT tumor suppressor homolog 4

ACCESSION = NP_443716
DEFINITION = WD repeat domain 10 isoform 4

ACCESSION = XP_935039
DEFINITION = PREDICTED: piccolo isoform 2

ACCESSION = NP_055731
DEFINITION = lemur tyrosine kinase 2

ACCESSION = XP_939204
DEFINITION = PREDICTED: piccolo isoform 3

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_976314
DEFINITION = acyl-CoA synthetase long-chain family member 5 isoform b

ACCESSION = NP_061142
DEFINITION = ATP-binding cassette, sub-family A , member 5

ACCESSION = NP_005679
DEFINITION = ATP-binding cassette, sub-family C, member 5 isoform 1

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_060732
DEFINITION = WD repeat domain 10 isoform 3

ACCESSION = NP_002323
DEFINITION = low density lipoprotein-related protein 1

ACCESSION = NP_001008844
DEFINITION = desmoplakin isoform II

ACCESSION = NP_006488
DEFINITION = hypermethylated in cancer 1

ACCESSION = NP_004399
DEFINITION = dynamin 1 isoform 1

ACCESSION = XP_290944
DEFINITION = PREDICTED: pleckstrin homology domain containing, family M (with RUN domain) member 2

ACCESSION = NP_061027
DEFINITION = low density lipoprotein-related protein 1B

ACCESSION = NP_443711
DEFINITION = WD repeat domain 10 isoform 1

ACCESSION = NP_003905
DEFINITION = cyclin A1

ACCESSION = XP_940803
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 7

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The pattern being searched is L[LMIF][DEN][LMIF][DEN].*f.d.f

73 matches found in protein
##############################
ACCESSION = NP_060808
DEFINITION = WD repeat domain 52

ACCESSION = NP_065965 XP_290768
DEFINITION = chromosome 17 open reading frame 27

ACCESSION = NP_002700
DEFINITION = protein phosphatase 1, catalytic subunit, beta isoform 1

ACCESSION = XP_939177
DEFINITION = PREDICTED: hypothetical protein LOC84162 isoform 5

ACCESSION = NP_065916
DEFINITION = DEAH (Asp-Glu-Ala-His) box polypeptide 36

ACCESSION = NP_597812
DEFINITION = sodium bicarbonate transporter 4 isoform c

ACCESSION = NP_055595
DEFINITION = cullin 7

ACCESSION = NP_055872
DEFINITION = MYC binding protein 2

ACCESSION = XP_948165
DEFINITION = PREDICTED: similar to CG4841-PA isoform 15

ACCESSION = NP_057373
DEFINITION = calcium binding protein 39

ACCESSION = NP_005329
DEFINITION = huntingtin interacting protein 1

ACCESSION = XP_941990
DEFINITION = PREDICTED: similar to CG4841-PA isoform 10

ACCESSION = NP_001061
DEFINITION = tubulin, gamma 1

ACCESSION = XP_939180
DEFINITION = PREDICTED: hypothetical protein LOC84162 isoform 6

ACCESSION = NP_085124
DEFINITION = dicer1

ACCESSION = XP_949034
DEFINITION = PREDICTED: similar to epidermal growth factor receptor pathway substrate 8-like protein 2 isoform 3

ACCESSION = XP_371706
DEFINITION = PREDICTED: hypothetical protein LOC84162 isoform 1

ACCESSION = NP_060024 XP_291055
DEFINITION = poly (ADP-ribose) polymerase family, member 14

ACCESSION = NP_149045
DEFINITION = protocadherin 15 precursor

ACCESSION = NP_002281
DEFINITION = laminin, alpha 4 precursor

ACCESSION = NP_002701
DEFINITION = protein phosphatase 1, catalytic subunit, gamma isoform

ACCESSION = NP_001026894
DEFINITION = calcium binding protein 39-like isoform 1

ACCESSION = NP_000935
DEFINITION = protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform (calcineurin A alpha)

ACCESSION = NP_003741
DEFINITION = eukaryotic translation initiation factor 3, subunit 10 theta, 150/170kDa

ACCESSION = XP_943674
DEFINITION = PREDICTED: similar to pleckstrin homology domain containing, family M (with RUN domain) member 1

ACCESSION = NP_004406
DEFINITION = desmoplakin isoform I

ACCESSION = NP_067019
DEFINITION = sodium bicarbonate transporter 4 isoform a

ACCESSION = NP_776152
DEFINITION = PDZ domain containing 8

ACCESSION = NP_003162
DEFINITION = suppressor of var1, 3-like 1

ACCESSION = NP_005752
DEFINITION = plexin C1

ACCESSION = NP_597813
DEFINITION = sodium bicarbonate transporter 4 isoform d

ACCESSION = NP_057346
DEFINITION = tubulin, epsilon 1

ACCESSION = NP_078898
DEFINITION = hypothetical protein LOC79675

ACCESSION = NP_525023
DEFINITION = ATP-binding cassette, sub-family A, member 6

ACCESSION = NP_996759
DEFINITION = protein phosphatase 1, catalytic subunit, beta isoform 1

ACCESSION = NP_004229 XP_376178
DEFINITION = thyroid hormone receptor interactor 12

ACCESSION = XP_949047
DEFINITION = PREDICTED: similar to epidermal growth factor receptor pathway substrate 8-like protein 2 isoform 6

ACCESSION = NP_001412
DEFINITION = E74-like factor 4 (ets domain transcription factor)

ACCESSION = NP_004438
DEFINITION = epidermal growth factor receptor pathway substrate 8

ACCESSION = NP_002709
DEFINITION = protein phosphatase 2, regulatory subunit B", alpha isoform 1

ACCESSION = NP_056029 XP_291291
DEFINITION = DDHD domain containing 2

ACCESSION = XP_948169
DEFINITION = PREDICTED: similar to CG4841-PA isoform 16

ACCESSION = NP_056109
DEFINITION = tripartite motif-containing 37 protein

ACCESSION = NP_803187
DEFINITION = dicer1

ACCESSION = NP_055434
DEFINITION = glutamate receptor KA1 precursor

ACCESSION = NP_057521
DEFINITION = tubulin, gamma 2

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_002699
DEFINITION = protein phosphatase 1, catalytic subunit, alpha isoform 1

ACCESSION = NP_201580
DEFINITION = sodium bicarbonate transporter 4 isoform b

ACCESSION = NP_055656 XP_498116
DEFINITION = synaptosomal-associated protein, 91kDa homolog

ACCESSION = NP_055071
DEFINITION = beta-1,3-N-acetylglucosaminyltransferase bGnT-3

ACCESSION = XP_949059
DEFINITION = PREDICTED: similar to epidermal growth factor receptor pathway substrate 8-like protein 2 isoform 10

ACCESSION = XP_948155
DEFINITION = PREDICTED: similar to CG4841-PA isoform 13

ACCESSION = NP_061908
DEFINITION = protocadherin 18 precursor

ACCESSION = NP_001005207
DEFINITION = tripartite motif-containing 37 protein

ACCESSION = XP_371474
DEFINITION = PREDICTED: plexin B2

ACCESSION = XP_943993
DEFINITION = PREDICTED: similar to epidermal growth factor receptor pathway substrate 8-like protein 2 isoform 2

ACCESSION = NP_055259
DEFINITION = gamma tubulin ring complex protein (76p gene)

ACCESSION = NP_001020948
DEFINITION = UDP-glucose ceramide glucosyltransferase-like 1 isoform 2

ACCESSION = XP_943338
DEFINITION = PREDICTED: similar to Plexin B2 precursor (MM1)

ACCESSION = NP_073609
DEFINITION = epidermal growth factor receptor pathway substrate 8-like protein 2

ACCESSION = NP_001008844
DEFINITION = desmoplakin isoform II

ACCESSION = NP_055904
DEFINITION = p53-associated parkin-like cytoplasmic protein

ACCESSION = NP_112187
DEFINITION = calcium binding protein 39-like isoform 2

ACCESSION = NP_064505
DEFINITION = UDP-glucose ceramide glucosyltransferase-like 1 isoform 1

ACCESSION = XP_290944
DEFINITION = PREDICTED: pleckstrin homology domain containing, family M (with RUN domain) member 2

ACCESSION = XP_939185
DEFINITION = PREDICTED: hypothetical protein LOC84162 isoform 7

ACCESSION = NP_001008709
DEFINITION = protein phosphatase 1, catalytic subunit, alpha isoform 3

ACCESSION = NP_443179
DEFINITION = heart alpha-kinase

ACCESSION = NP_003905
DEFINITION = cyclin A1

ACCESSION = NP_004949
DEFINITION = FK506 binding protein 12-rapamycin associated protein 1

ACCESSION = NP_031373
DEFINITION = adaptor-related protein complex 4, epsilon 1 subunit

ACCESSION = NP_005642
DEFINITION = tryptophan 2,3-dioxygenase

****
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The pattern being searched is L[LMIF][DEN][LMIF][DEN].*w..[fw].[de]

63 matches found in protein
##########
ACCESSION = NP_065164 XP_041116
DEFINITION = transmembrane protein 63C

ACCESSION = NP_056193 XP_044546
DEFINITION = vacuolar protein sorting 13D isoform 1

ACCESSION = NP_149033
DEFINITION = microfilament and actin filament cross-linker protein isoform b

ACCESSION = NP_149062
DEFINITION = nesprin 1 isoform longer

ACCESSION = NP_000338
DEFINITION = spectrin beta isoform b

ACCESSION = NP_002700
DEFINITION = protein phosphatase 1, catalytic subunit, beta isoform 1

ACCESSION = NP_055595
DEFINITION = cullin 7

ACCESSION = XP_942900
DEFINITION = PREDICTED: similar to dynein, cytoplasmic, heavy chain 2 isoform 3

ACCESSION = NP_001363
DEFINITION = dynein, axonemal, heavy polypeptide 9 isoform 2

ACCESSION = NP_842565
DEFINITION = spectrin, beta, non-erythrocytic 1 isoform 2

ACCESSION = NP_065779
DEFINITION = family with sequence similarity 62 (C2 domain containing) member B

ACCESSION = NP_690043
DEFINITION = angiotensin I converting enzyme isoform 2 precursor

ACCESSION = NP_536724
DEFINITION = cell division cycle 91-like 1 protein

ACCESSION = NP_065872
DEFINITION = vacuolar protein sorting 13C protein isoform 2A

ACCESSION = NP_001018098
DEFINITION = vacuolar protein sorting 13C protein isoform 2B

ACCESSION = NP_002701
DEFINITION = protein phosphatase 1, catalytic subunit, gamma isoform

ACCESSION = XP_166254
DEFINITION = PREDICTED: odz, odd Oz/ten-m homolog 4

ACCESSION = NP_078962
DEFINITION = tubulin tyrosine ligase-like family, member 7

ACCESSION = NP_055995
DEFINITION = spectrin repeat containing, nuclear envelope 2 isoform a

ACCESSION = XP_943080
DEFINITION = PREDICTED: similar to kinesin family member 27 isoform 2

ACCESSION = NP_690044
DEFINITION = angiotensin I converting enzyme isoform 3 precursor

ACCESSION = NP_689914
DEFINITION = ATP binding cassette, sub-family A (ABC1), member 13

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = XP_377696
DEFINITION = PREDICTED: similar to carbonic anhydrase 15

ACCESSION = XP_943924
DEFINITION = PREDICTED: similar to Mucin-5B precursor (Mucin 5 subtype B, tracheobronchial) (High molecular weight salivary mucin MG1) (Sublingual gland mucin)

ACCESSION = NP_057645
DEFINITION = apoptosis regulator

ACCESSION = NP_001362
DEFINITION = dynein, axonemal, heavy polypeptide 8

ACCESSION = XP_945556
DEFINITION = PREDICTED: similar to odd Oz/ten-m homolog 4

ACCESSION = NP_996759
DEFINITION = protein phosphatase 1, catalytic subunit, beta isoform 1

ACCESSION = NP_057733
DEFINITION = ABT1-associated protein

ACCESSION = NP_060550
DEFINITION = vacuolar protein sorting 13C protein isoform 1B

ACCESSION = NP_002215
DEFINITION = inositol 1,4,5-triphosphate receptor, type 3

ACCESSION = NP_006210
DEFINITION = phosphoinositide-3-kinase, catalytic, beta polypeptide

ACCESSION = NP_002214
DEFINITION = inositol 1,4,5-triphosphate receptor, type 2

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_114128
DEFINITION = RALBP1 associated Eps domain containing 1

ACCESSION = NP_065870 XP_371832
DEFINITION = hypothetical protein LOC57579

ACCESSION = XP_039877
DEFINITION = PREDICTED: mucin 5, subtype B, tracheobronchial

ACCESSION = NP_899236
DEFINITION = dystonin isoform 1

ACCESSION = NP_002699
DEFINITION = protein phosphatase 1, catalytic subunit, alpha isoform 1

ACCESSION = NP_005090
DEFINITION = ADAM metallopeptidase with thrombospondin type 1 motif, 4 preproprotein

ACCESSION = NP_001360
DEFINITION = dynein, axonemal, heavy polypeptide 5

ACCESSION = XP_948598
DEFINITION = PREDICTED: similar to kinesin family member 27 isoform 4

ACCESSION = NP_060626
DEFINITION = vacuolar protein sorting 13D isoform 2

ACCESSION = NP_001020029
DEFINITION = spectrin beta isoform a

ACCESSION = XP_948603
DEFINITION = PREDICTED: similar to kinesin family member 27 isoform 5

ACCESSION = NP_079410
DEFINITION = chromodomain helicase DNA binding protein 9

ACCESSION = NP_065077
DEFINITION = solute carrier family 24 (sodium/potassium/calcium exchanger), member 2

ACCESSION = NP_060154
DEFINITION = vacuolar protein sorting 13C protein isoform 1A

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_002323
DEFINITION = low density lipoprotein-related protein 1

ACCESSION = NP_892006
DEFINITION = nesprin 1 longest

ACCESSION = NP_000780
DEFINITION = angiotensin I converting enzyme isoform 1 precursor

ACCESSION = XP_370652
DEFINITION = PREDICTED: dynein, cytoplasmic, heavy polypeptide 2 isoform 1

ACCESSION = NP_982271
DEFINITION = synaptojanin 1 isoform b

ACCESSION = XP_942832
DEFINITION = PREDICTED: similar to carbonic anhydrase 15

ACCESSION = NP_055904
DEFINITION = p53-associated parkin-like cytoplasmic protein

ACCESSION = NP_689829
DEFINITION = tripartite motif-containing 42

ACCESSION = NP_061027
DEFINITION = low density lipoprotein-related protein 1B

ACCESSION = NP_055524 XP_291018
DEFINITION = ubiquitin specific protease 34

ACCESSION = NP_001008709
DEFINITION = protein phosphatase 1, catalytic subunit, alpha isoform 3

ACCESSION = NP_114141
DEFINITION = hemicentin 1

ACCESSION = NP_003119
DEFINITION = spectrin, beta, non-erythrocytic 1 isoform 1

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The pattern being searched is L[LMIF][DEN][LMIF][DEN].*DDGLDEAFSRLAQSRT

One match found

##############################
ACCESSION = NP_056442
DEFINITION = low density lipoprotein receptor adaptor protein 1

****
****
The pattern being searched is L[LMIF][DEN][LMIF][DEN].*L[LMIF][DEN][LMIF][DEN]

80 matches found in protein
##############################
ACCESSION = NP_008877
DEFINITION = spectrin, beta, non-erythrocytic 2

ACCESSION = XP_949035
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 14

ACCESSION = NP_060808
DEFINITION = WD repeat domain 52

ACCESSION = NP_079146
DEFINITION = DEP domain containing 2 isoform a

ACCESSION = NP_065965 XP_290768
DEFINITION = chromosome 17 open reading frame 27

ACCESSION = XP_939711
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 9

ACCESSION = NP_742093
DEFINITION = sperm-associated cation channel 2 isoform 2

ACCESSION = XP_939708
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 6

ACCESSION = NP_058633
DEFINITION = polymerase (DNA-directed), alpha

ACCESSION = NP_055872
DEFINITION = MYC binding protein 2

ACCESSION = NP_071407
DEFINITION = cadherin related 23 isoform 1 precursor

ACCESSION = NP_004696
DEFINITION = protein-kinase, interferon-inducible double stranded RNA dependent inhibitor, repressor of (P58 repressor)

ACCESSION = NP_573444
DEFINITION = epidermal growth factor receptor pathway substrate 8-like protein 3 isoform b

ACCESSION = NP_002171
DEFINITION = immunoglobulin mu binding protein 2

ACCESSION = NP_059447
DEFINITION = major vault protein

ACCESSION = XP_939707
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 5

ACCESSION = NP_085124
DEFINITION = dicer1

ACCESSION = NP_006297
DEFINITION = SMC1 structural maintenance of chromosomes 1-like 1

ACCESSION = NP_877496
DEFINITION = engulfment and cell motility 2

ACCESSION = NP_060914
DEFINITION = pyruvate dehydrogenase phosphatase precursor

ACCESSION = NP_757367
DEFINITION = sperm associated antigen 1

ACCESSION = NP_065872
DEFINITION = vacuolar protein sorting 13C protein isoform 2A

ACCESSION = XP_946300
DEFINITION = PREDICTED: similar to FRAS1-related extracellular matrix protein 2 precursor (ECM3 homolog)

ACCESSION = NP_001018098
DEFINITION = vacuolar protein sorting 13C protein isoform 2B

ACCESSION = XP_949050
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 18

ACCESSION = XP_934977
DEFINITION = PREDICTED: FRAS1 related extracellular matrix protein 2

ACCESSION = NP_005210
DEFINITION = diaphanous 1

ACCESSION = NP_005106
DEFINITION = major vault protein

ACCESSION = NP_071373 XP_371779
DEFINITION = transposon-derived Buster3 transposase-like

ACCESSION = NP_065789 XP_291015
DEFINITION = kinase D-interacting substance of 220 kDa

ACCESSION = NP_776152
DEFINITION = PDZ domain containing 8

ACCESSION = NP_003162
DEFINITION = suppressor of var1, 3-like 1

ACCESSION = NP_733468
DEFINITION = cancer susceptibility candidate 5 isoform 1

ACCESSION = XP_939709
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 7

ACCESSION = NP_004923
DEFINITION = cadherin 6, type 2 preproprotein

ACCESSION = NP_149132 XP_027237
DEFINITION = mitogen-activated protein kinase kinase kinase 9

ACCESSION = NP_065928 XP_209041
DEFINITION = protein similar to dynein

ACCESSION = NP_001118
DEFINITION = adaptor-related protein complex 1 beta 1 subunit isoform a

ACCESSION = NP_078953
DEFINITION = hypothetical protein LOC79730

ACCESSION = XP_949040
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 15

ACCESSION = NP_060387
DEFINITION = chromosome 14 open reading frame 10

ACCESSION = NP_055684
DEFINITION = IQ motif and Sec7 domain 1

ACCESSION = NP_003728
DEFINITION = dachsous 1 precursor

ACCESSION = XP_944006
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 10

ACCESSION = NP_620641
DEFINITION = epidermal growth factor receptor pathway substrate 8-like protein 3 isoform a

ACCESSION = NP_057733
DEFINITION = ABT1-associated protein

ACCESSION = XP_949019
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 11

ACCESSION = NP_078802
DEFINITION = epidermal growth factor receptor pathway substrate 8-like protein 3 isoform c

ACCESSION = NP_060550
DEFINITION = vacuolar protein sorting 13C protein isoform 1B

ACCESSION = NP_037523
DEFINITION = dimethylglycine dehydrogenase precursor

ACCESSION = NP_733792
DEFINITION = mitochondrial elongation factor G2 isoform 2

ACCESSION = NP_005503
DEFINITION = leucine rich repeat containing 32 precursor

ACCESSION = XP_939703
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 2

ACCESSION = NP_006210
DEFINITION = phosphoinositide-3-kinase, catalytic, beta polypeptide

ACCESSION = XP_935400
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 1

ACCESSION = NP_803187
DEFINITION = dicer1

ACCESSION = NP_003105
DEFINITION = sperm associated antigen 1

ACCESSION = NP_071369
DEFINITION = engulfment and cell motility 2

ACCESSION = NP_075463
DEFINITION = hypothetical protein CG003

ACCESSION = NP_003117
DEFINITION = spectrin, alpha, erythrocytic 1 (elliptocytosis 2)

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = XP_949027
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 13

ACCESSION = NP_055615
DEFINITION = engulfment and cell motility 1 isoform 1

ACCESSION = NP_065801
DEFINITION = exportin 5

ACCESSION = NP_056371
DEFINITION = signal-induced proliferation-associated 1 like 1

ACCESSION = XP_949042
DEFINITION = PREDICTED: similar to ral guanine nucleotide dissociation stimulator-like 3 isoform 16

ACCESSION = NP_060154
DEFINITION = vacuolar protein sorting 13C protein isoform 1A

ACCESSION = NP_036273
DEFINITION = DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26

ACCESSION = XP_093839
DEFINITION = PREDICTED: hypothetical protein LOC23045 isoform 1

ACCESSION = XP_939706
DEFINITION = PREDICTED: ral guanine nucleotide dissociation stimulator-like 3 isoform 4

ACCESSION = NP_004265
DEFINITION = A-kinase anchor protein 6

ACCESSION = NP_001273
DEFINITION = adaptor-related protein complex 2, beta 1 subunit isoform b

ACCESSION = NP_473361
DEFINITION = sperm-associated cation channel 2 isoform 1

ACCESSION = NP_001025177
DEFINITION = adaptor-related protein complex 2, beta 1 subunit isoform a

ACCESSION = NP_663782
DEFINITION = adaptor-related protein complex 1 beta 1 subunit isoform b

ACCESSION = NP_653091
DEFINITION = cancer susceptibility candidate 5 isoform 2

ACCESSION = XP_946679
DEFINITION = PREDICTED: similar to CG32045-PB, isoform B isoform 5

ACCESSION = NP_115756
DEFINITION = mitochondrial elongation factor G2 isoform 1

ACCESSION = NP_060395
DEFINITION = hypothetical protein LOC55667

ACCESSION = NP_573403
DEFINITION = engulfment and cell motility 2

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****
The pattern being searched is L[LMIF][DEN][LMIF][DEN].*npf

48 matches found in protein
##########
ACCESSION = NP_060109
DEFINITION = dachsous 2 isoform 1

ACCESSION = NP_872346 XP_498462 XP_499592
DEFINITION = hypothetical protein LOC203522

ACCESSION = NP_000149
DEFINITION = glucan (1,4-alpha-), branching enzyme 1 (glycogen branching enzyme)

ACCESSION = XP_942900
DEFINITION = PREDICTED: similar to dynein, cytoplasmic, heavy chain 2 isoform 3

ACCESSION = NP_689488
DEFINITION = hypothetical protein LOC92104

ACCESSION = NP_620309
DEFINITION = B-cell lymphoma 6 protein

ACCESSION = NP_065791
DEFINITION = ankyrin repeat and FYVE domain containing 1 isoform 2

ACCESSION = NP_060653
DEFINITION = sno, strawberry notch homolog 1

ACCESSION = NP_057336
DEFINITION = baculoviral IAP repeat-containing 6

ACCESSION = NP_001008660
DEFINITION = phosphatidylinositol-binding clathrin assembly protein isoform 2

ACCESSION = NP_065872
DEFINITION = vacuolar protein sorting 13C protein isoform 2A

ACCESSION = XP_941767
DEFINITION = PREDICTED: similar to Suppressor Of Clr family member (soc-2)

ACCESSION = NP_001018098
DEFINITION = vacuolar protein sorting 13C protein isoform 2B

ACCESSION = NP_056081 XP_030524
DEFINITION = Na+/H+ exchanger isoform 8

ACCESSION = XP_166254
DEFINITION = PREDICTED: odz, odd Oz/ten-m homolog 4

ACCESSION = XP_943674
DEFINITION = PREDICTED: similar to pleckstrin homology domain containing, family M (with RUN domain) member 1

ACCESSION = XP_931283
DEFINITION = PREDICTED: similar to Leucine-rich repeat-containing protein 1 (LAP and no PDZ protein) (LANO adapter protein)

ACCESSION = NP_001697
DEFINITION = B-cell lymphoma 6 protein

ACCESSION = NP_079039
DEFINITION = WD repeat domain 78 isoform 1

ACCESSION = NP_005348
DEFINITION = hormone-sensitive lipase

ACCESSION = NP_057460
DEFINITION = ankyrin repeat and FYVE domain containing 1 isoform 1

ACCESSION = NP_877439 XP_294213 XP_353565
DEFINITION = putative binding protein 7a5

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = XP_943924
DEFINITION = PREDICTED: similar to Mucin-5B precursor (Mucin 5 subtype B, tracheobronchial) (High molecular weight salivary mucin MG1) (Sublingual gland mucin)

ACCESSION = NP_009097
DEFINITION = phosphatidylinositol-binding clathrin assembly protein isoform 1

ACCESSION = XP_945556
DEFINITION = PREDICTED: similar to odd Oz/ten-m homolog 4

ACCESSION = NP_058632
DEFINITION = ubinuclein 1

ACCESSION = NP_055811 XP_042635
DEFINITION = phospholipase C-like 3

ACCESSION = NP_060550
DEFINITION = vacuolar protein sorting 13C protein isoform 1B

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = NP_006210
DEFINITION = phosphoinositide-3-kinase, catalytic, beta polypeptide

ACCESSION = NP_056986
DEFINITION = E3 ubiquitin protein ligase, HECT domain containing, 1

ACCESSION = XP_941327
DEFINITION = PREDICTED: similar to E3 ubiquitin protein ligase, HECT domain containing, 1

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = XP_039877
DEFINITION = PREDICTED: mucin 5, subtype B, tracheobronchial

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = XP_949600
DEFINITION = PREDICTED: similar to Tubulin gamma-1 chain (Gamma-1 tubulin) (Gamma-tubulin complex component 1) (GCP-1) isoform 3

ACCESSION = NP_203744
DEFINITION = molecule interacting with Rab13

ACCESSION = NP_689730
DEFINITION = hypothetical protein LOC150737

ACCESSION = NP_060154
DEFINITION = vacuolar protein sorting 13C protein isoform 1A

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_036273
DEFINITION = DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26

ACCESSION = NP_955452 NP_054844
DEFINITION = DNA polymerase theta

ACCESSION = XP_370652
DEFINITION = PREDICTED: dynein, cytoplasmic, heavy polypeptide 2 isoform 1

ACCESSION = XP_290944
DEFINITION = PREDICTED: pleckstrin homology domain containing, family M (with RUN domain) member 2

ACCESSION = NP_055524 XP_291018
DEFINITION = ubiquitin specific protease 34

ACCESSION = NP_005305
DEFINITION = gastrin-releasing peptide receptor

****
****
The pattern being searched is npf.*dp[fw]

99 matches found in protein
##############################
ACCESSION = NP_055963
DEFINITION = PAS domain containing serine/threonine kinase

ACCESSION = NP_579899
DEFINITION = myoferlin isoform b

ACCESSION = NP_115515
DEFINITION = ankyrin repeat domain 27 (VPS9 domain)

ACCESSION = NP_055680
DEFINITION = chromosome condensation-related SMC-associated protein 1

ACCESSION = NP_005622
DEFINITION = smoothened

ACCESSION = NP_006274
DEFINITION = transforming, acidic coiled-coil containing protein 1

ACCESSION = NP_008928
DEFINITION = transforming, acidic coiled-coil containing protein 2 isoform d

ACCESSION = XP_943656
DEFINITION = PREDICTED: similar to Zinc finger protein 292 isoform 4

ACCESSION = NP_000009
DEFINITION = acyl-Coenzyme A dehydrogenase, very long chain isoform 1 precursor

ACCESSION = NP_055483
DEFINITION = GREB1 protein isoform a

ACCESSION = NP_066921
DEFINITION = calcium channel, voltage-dependent, alpha 1H subunit isoform a

ACCESSION = NP_065997 XP_497076
DEFINITION = hypothetical protein LOC57706 isoform 1

ACCESSION = XP_945898
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 12

ACCESSION = NP_006136
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 1

ACCESSION = NP_003595
DEFINITION = insulin receptor substrate 4

ACCESSION = NP_055927
DEFINITION = microtubule associated serine/threonine kinase 2

ACCESSION = XP_941081
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 11

ACCESSION = XP_941078
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 8

ACCESSION = NP_060653
DEFINITION = sno, strawberry notch homolog 1

ACCESSION = NP_001008220
DEFINITION = amylase, alpha 1C; salivary precursor

ACCESSION = XP_048070
DEFINITION = PREDICTED: zinc finger protein 292 isoform 1

ACCESSION = XP_945671
DEFINITION = PREDICTED: hypothetical protein XP_945671

ACCESSION = XP_942211
DEFINITION = PREDICTED: similar to double homeobox 4c

ACCESSION = NP_004029
DEFINITION = amylase, alpha 1A; salivary precursor

ACCESSION = NP_612563
DEFINITION = B-cell CLL/lymphoma 11A isoform 5

ACCESSION = NP_075049
DEFINITION = B-cell CLL/lymphoma 11B isoform 2

ACCESSION = NP_652761
DEFINITION = A-kinase anchor protein 11 isoform 2

ACCESSION = XP_086287
DEFINITION = PREDICTED: similar to protein tyrosine phosphatase, receptor type, V

ACCESSION = NP_851607
DEFINITION = SCY1-like 3 isoform 2

ACCESSION = NP_065710
DEFINITION = tweety 1 isoform 1

ACCESSION = NP_055726
DEFINITION = AP2 associated kinase 1

ACCESSION = NP_002963 XP_037447
DEFINITION = SET binding factor 1 isoform a

ACCESSION = NP_942595
DEFINITION = BMP-2 inducible kinase isoform a

ACCESSION = NP_004227
DEFINITION = COP9 constitutive photomorphogenic homolog subunit 2

ACCESSION = XP_943674
DEFINITION = PREDICTED: similar to pleckstrin homology domain containing, family M (with RUN domain) member 1

ACCESSION = NP_996742
DEFINITION = transforming, acidic coiled-coil containing protein 2 isoform c

ACCESSION = NP_036586
DEFINITION = T-cell lymphoma invasion and metastasis 2 isoform a

ACCESSION = XP_939215
DEFINITION = PREDICTED: piccolo isoform 6

ACCESSION = NP_001501
DEFINITION = glutamate receptor, ionotropic, delta 2

ACCESSION = XP_946113
DEFINITION = PREDICTED: similar to protein tyrosine phosphatase, receptor type, V

ACCESSION = NP_996743
DEFINITION = transforming, acidic coiled-coil containing protein 2 isoform b

ACCESSION = NP_055621 XP_048462
DEFINITION = RUN and SH3 domain containing 2

ACCESSION = NP_001029031
DEFINITION = acyl-Coenzyme A dehydrogenase, very long chain isoform 2 precursor

ACCESSION = XP_932914
DEFINITION = PREDICTED: similar to Gamma-tubulin complex component 3 (GCP-3) (Spindle pole body protein Spc98 homolog) (hSpc98) (hGCP3) (h104p)

ACCESSION = NP_001010972
DEFINITION = zyxin

ACCESSION = XP_377445
DEFINITION = PREDICTED: similar to double homeobox 4c

ACCESSION = NP_006039
DEFINITION = ubiquitination factor E4B

ACCESSION = NP_877439 XP_294213 XP_353565
DEFINITION = putative binding protein 7a5

ACCESSION = NP_001010927
DEFINITION = T-cell lymphoma invasion and metastasis 2 isoform b

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = NP_996744
DEFINITION = transforming, acidic coiled-coil containing protein 2 isoform a

ACCESSION = XP_949903
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 16

ACCESSION = NP_612808
DEFINITION = B-cell CLL/lymphoma 11B isoform 1

ACCESSION = NP_001004051
DEFINITION = G protein-coupled receptor associated sorting protein 2

ACCESSION = NP_001005407
DEFINITION = calcium channel, voltage-dependent, alpha 1H subunit isoform b

ACCESSION = NP_060466
DEFINITION = de-etiolated 1

ACCESSION = NP_004749
DEFINITION = peripheral benzodiazepine receptor-associated protein 1

ACCESSION = XP_495939
DEFINITION = PREDICTED: hypothetical protein LOC57666

ACCESSION = NP_001008222
DEFINITION = amylase, alpha 1A; salivary precursor

ACCESSION = XP_942238
DEFINITION = PREDICTED: similar to myoferlin isoform b

ACCESSION = NP_055811 XP_042635
DEFINITION = phospholipase C-like 3

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = NP_001008219
DEFINITION = amylase, alpha 1B; salivary precursor

ACCESSION = NP_005586
DEFINITION = nuclear factor I/A

ACCESSION = XP_949889
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 13

ACCESSION = XP_097977
DEFINITION = PREDICTED: hypothetical protein XP_097977

ACCESSION = NP_001010870 XP_166443
DEFINITION = tudor domain containing 6

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_116250
DEFINITION = serine active site containing 1

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_689804
DEFINITION = hypothetical protein LOC160762

ACCESSION = XP_094074
DEFINITION = PREDICTED: similar to FRAS1-related extracellular matrix protein 3 precursor

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_036547
DEFINITION = RAD54 homolog B

ACCESSION = XP_941521
DEFINITION = PREDICTED: similar to autism susceptibility candidate 2

ACCESSION = XP_935039
DEFINITION = PREDICTED: piccolo isoform 2

ACCESSION = NP_075044
DEFINITION = B-cell CLL/lymphoma 11A isoform 1

ACCESSION = NP_653308
DEFINITION = prominin 2

ACCESSION = NP_079097 XP_496529
DEFINITION = hypothetical protein LOC79879

ACCESSION = NP_056018 XP_371309
DEFINITION = hypothetical protein LOC23248

ACCESSION = XP_291671
DEFINITION = PREDICTED: similar to Matrin-3

ACCESSION = XP_939204
DEFINITION = PREDICTED: piccolo isoform 3

ACCESSION = XP_946603
DEFINITION = PREDICTED: similar to DnaJ homolog subfamily B member 5 (Heat shock protein Hsp40-3) (Heat shock protein cognate 40) (Hsc40) (Hsp40-2) isoform 2

ACCESSION = NP_038479
DEFINITION = myoferlin isoform a

ACCESSION = NP_060484 NP_612562
DEFINITION = B-cell CLL/lymphoma 11A isoform 2

ACCESSION = NP_056040 XP_041018
DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_000690
DEFINITION = amylase, alpha 2A; pancreatic precursor

ACCESSION = NP_057332
DEFINITION = A-kinase anchor protein 11 isoform 1

ACCESSION = NP_612446
DEFINITION = G protein-coupled receptor associated sorting protein 2

ACCESSION = XP_290944
DEFINITION = PREDICTED: pleckstrin homology domain containing, family M (with RUN domain) member 2

ACCESSION = XP_944430
DEFINITION = PREDICTED: similar to Matrin-3

ACCESSION = NP_036398
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 5

ACCESSION = NP_066188
DEFINITION = amylase, alpha 2B; pancreatic precursor

ACCESSION = NP_004779
DEFINITION = ubiquitination factor E4A

ACCESSION = NP_003452
DEFINITION = zyxin

ACCESSION = XP_940803
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 7

ACCESSION = NP_008965
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 4

ACCESSION = NP_071897
DEFINITION = fibrosin 1

****
****
The pattern being searched is npf.*f.d.f

38 matches found in protein
##############################

ACCESSION = NP_579899
DEFINITION = myoferlin isoform b

ACCESSION = NP_612149
DEFINITION = ataxia telangiectasia mutated protein isoform 2

ACCESSION = NP_473454
DEFINITION = DNA-dependent protein kinase catalytic subunit-interacting protein 3

ACCESSION = NP_058633
DEFINITION = polymerase (DNA-directed), alpha

ACCESSION = NP_787072
DEFINITION = exocyst complex 84-kDa subunit

ACCESSION = NP_000042
DEFINITION = ataxia telangiectasia mutated protein isoform 1

ACCESSION = NP_055950 XP_371954 XP_374530
DEFINITION = nucleoporin 205kDa

ACCESSION = NP_004295
DEFINITION = anaplastic lymphoma kinase Ki-1

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_997404
DEFINITION = reticulon 4 isoform E

ACCESSION = NP_001030
DEFINITION = sodium channel, nonvoltage-gated 1, gamma

ACCESSION = NP_055621 XP_048462
DEFINITION = RUN and SH3 domain containing 2

ACCESSION = NP_631905
DEFINITION = striated muscle activator of Rho-dependent signaling

ACCESSION = NP_060647
DEFINITION = Nedd4 binding protein 2

ACCESSION = NP_006039
DEFINITION = ubiquitination factor E4B

ACCESSION = NP_055426
DEFINITION = MDN1, midasin homolog

ACCESSION = NP_060466
DEFINITION = de-etiolated 1

ACCESSION = XP_941345
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = XP_940697
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = NP_001032
DEFINITION = sucrase-isomaltase (alpha-glucosidase)

ACCESSION = XP_944522
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_057688
DEFINITION = jumonji domain containing 1B

ACCESSION = NP_006589
DEFINITION = solute carrier family 12 (potassium/chloride transporters), member 7

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_003256
DEFINITION = toll-like receptor 3

ACCESSION = NP_006827 XP_045792
DEFINITION = GCN1 general control of amino-acid synthesis 1-like 1

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_006374
DEFINITION = DNA-dependent protein kinase catalytic subunit-interacting protein 2

ACCESSION = NP_065393
DEFINITION = reticulon 4 isoform A

ACCESSION = NP_056167
DEFINITION = protein O-fucosyltransferase 1 isoform 1 precursor

ACCESSION = NP_038479
DEFINITION = myoferlin isoform a

ACCESSION = NP_689483
DEFINITION = hypothetical protein FLJ34922

ACCESSION = NP_758436
DEFINITION = protein O-fucosyltransferase 1 isoform 2 precursor

ACCESSION = XP_931230
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_056031
DEFINITION = hypothetical protein LOC23262

ACCESSION = XP_945884
DEFINITION = PREDICTED: similar to Serine-protein kinase ATM (Ataxia telangiectasia mutated) (A-T, mutated)

ACCESSION = NP_056099
DEFINITION = KIAA0467 protein

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The pattern being searched is npf.*W..[FW].[DE]

44 matches found in protein
##########
ACCESSION = NP_055111
DEFINITION = calpain 7

ACCESSION = NP_055680
DEFINITION = chromosome condensation-related SMC-associated protein 1

ACCESSION = NP_112219
DEFINITION = ADAM metallopeptidase with thrombospondin type 1 motif, 10 preproprotein

ACCESSION = NP_891555
DEFINITION = fms-related tyrosine kinase 4 isoform 1

ACCESSION = NP_597705 XP_062545
DEFINITION = hypothetical protein LOC121256

ACCESSION = XP_942900
DEFINITION = PREDICTED: similar to dynein, cytoplasmic, heavy chain 2 isoform 3

ACCESSION = NP_001019980
DEFINITION = retinoic acid receptor, alpha isoform b

ACCESSION = NP_002011
DEFINITION = fms-related tyrosine kinase 4 isoform 2

ACCESSION = NP_079426
DEFINITION = threonyl-tRNA synthetase-like 1

ACCESSION = NP_005457
DEFINITION = mediator of RNA polymerase II transcription, subunit 6 homolog

ACCESSION = XP_941326
DEFINITION = PREDICTED: similar to Maltase-glucoamylase, intestinal

ACCESSION = NP_851607
DEFINITION = SCY1-like 3 isoform 2

ACCESSION = NP_003585
DEFINITION = transcription termination factor, RNA polymerase II

ACCESSION = NP_066988
DEFINITION = BMP/retinoic acid-inducible neural-specific protein 2

ACCESSION = XP_166254
DEFINITION = PREDICTED: odz, odd Oz/ten-m homolog 4

ACCESSION = XP_936549

DEFINITION = PREDICTED: odz, odd Oz/ten-m homolog 2 isoform 3

ACCESSION = XP_047995
DEFINITION = PREDICTED: odz, odd Oz/ten-m homolog 2 isoform 1

ACCESSION = NP_055336
DEFINITION = SH3-domain binding protein 4

ACCESSION = NP_004823
DEFINITION = glutathione-S-transferase omega 1

ACCESSION = NP_851320
DEFINITION = intimal thickness-related receptor

ACCESSION = NP_055433
DEFINITION = deleted in bladder cancer 1

ACCESSION = XP_943924
DEFINITION = PREDICTED: similar to Mucin-5B precursor (Mucin 5 subtype B, tracheobronchial) (High molecular weight salivary mucin MG1) (Sublingual gland mucin)

ACCESSION = NP_950252 XP_290915
DEFINITION = family with sequence similarity 5, member C

ACCESSION = NP_922932 NP_055088 XP_293904
DEFINITION = ADAM metallopeptidase with thrombospondin type 1 motif, 6 preproprotein

ACCESSION = XP_945556
DEFINITION = PREDICTED: similar to odd Oz/ten-m homolog 4

ACCESSION = XP_948728
DEFINITION = PREDICTED: hypothetical protein XP_948728

ACCESSION = NP_006210
DEFINITION = phosphoinositide-3-kinase, catalytic, beta polypeptide

ACCESSION = NP_001010870 XP_166443
DEFINITION = tudor domain containing 6

ACCESSION = NP_004659
DEFINITION = maltase-glucoamylase

ACCESSION = NP_057688
DEFINITION = jumonji domain containing 1B

ACCESSION = NP_006589
DEFINITION = solute carrier family 12 (potassium/chloride transporters), member 7

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = NP_937784 XP_370857
DEFINITION = glucosidase, alpha; neutral C

ACCESSION = XP_039877
DEFINITION = PREDICTED: mucin 5, subtype B, tracheobronchial

ACCESSION = XP_947301
DEFINITION = PREDICTED: similar to odd Oz/ten-m homolog 2 isoform 4

ACCESSION = NP_001009944
DEFINITION = polycystin 1 isoform 1 precursor

ACCESSION = NP_776297
DEFINITION = chloride channel 3 isoform e

ACCESSION = NP_612409 XP_049384 XP_374590
DEFINITION = nucleolar protein with MIF4G domain 1

ACCESSION = NP_060250 XP_098762
DEFINITION = chromodomain helicase DNA binding protein 7

ACCESSION = XP_950881
DEFINITION = PREDICTED: similar to odd Oz/ten-m homolog 2 isoform 6

ACCESSION = NP_000287
DEFINITION = polycystin 1 isoform 2 precursor

ACCESSION = XP_370652
DEFINITION = PREDICTED: dynein, cytoplasmic, heavy polypeptide 2 isoform 1

ACCESSION = NP_001820
DEFINITION = chloride channel 3 isoform b

ACCESSION = NP_038474
DEFINITION = makorin, ring finger protein, 1

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****
The pattern being searched is npf.*DDGLDEAFSRLAQSRT

No matches found

****
****
The pattern being searched is npf.*L[LMIF][DEN][LMIF][DEN]

55 matches found in protein
##########
ACCESSION = NP_060109
DEFINITION = dachsous 2 isoform 1

ACCESSION = NP_055477 XP_376007
DEFINITION = DEP domain containing 5 isoform 1

ACCESSION = XP_945898
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 12

ACCESSION = NP_058633
DEFINITION = polymerase (DNA-directed), alpha

ACCESSION = XP_941081
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 11

ACCESSION = XP_941078
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 8

ACCESSION = XP_944389
DEFINITION = PREDICTED: similar to MAX-interacting protein isoform 5

ACCESSION = NP_055950 XP_371954 XP_374530
DEFINITION = nucleoporin 205kDa

ACCESSION = XP_940748
DEFINITION = PREDICTED: similar to Rap guanine nucleotide exchange factor 5 (Guanine nucleotide exchange factor for Rap1) (Related to Epac) (Repac) (M-Ras-regulated Rap GEF) (MR-GEF) isoform 6

ACCESSION = NP_115832
DEFINITION = protocadherin 7 isoform b precursor

ACCESSION = NP_003820
DEFINITION = multiple PDZ domain protein

ACCESSION = NP_055558 XP_291106
DEFINITION = hypothetical protein LOC9778

ACCESSION = NP_114072
DEFINITION = frizzled 8

ACCESSION = NP_004227
DEFINITION = COP9 constitutive photomorphogenic homolog subunit 2

ACCESSION = NP_003772
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = XP_939215
DEFINITION = PREDICTED: piccolo isoform 6

ACCESSION = NP_060434
DEFINITION = solute carrier family 30 (zinc transporter), member 6

ACCESSION = NP_055416
DEFINITION = EH-domain containing 2

ACCESSION = NP_775792
DEFINITION = hypothetical protein LOC158401

ACCESSION = XP_933695
DEFINITION = PREDICTED: Rap guanine nucleotide exchange factor (GEF) 5 isoform 1

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = XP_949903

DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 16

ACCESSION = NP_001004051
DEFINITION = G protein-coupled receptor associated sorting protein 2

ACCESSION = NP_055426
DEFINITION = MDN1, midasin homolog

ACCESSION = NP_149358
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = XP_937813
DEFINITION = PREDICTED: MAX dimerization protein 5 isoform 2

ACCESSION = NP_060137
DEFINITION = hypothetical protein LOC55610 isoform a

ACCESSION = NP_149359
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = XP_942238
DEFINITION = PREDICTED: similar to myoferlin isoform b

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = XP_031689
DEFINITION = PREDICTED: MAX dimerization protein 5 isoform 1

ACCESSION = XP_942937
DEFINITION = PREDICTED: similar to Rap guanine nucleotide exchange factor 5 (Guanine nucleotide exchange factor for Rap1) (Related to Epac) (Repac) (M-Ras-regulated Rap GEF) (MR-GEF) isoform 11

ACCESSION = NP_006210
DEFINITION = phosphoinositide-3-kinase, catalytic, beta polypeptide

ACCESSION = XP_949889
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 13

ACCESSION = NP_115833
DEFINITION = protocadherin 7 isoform c precursor

ACCESSION = XP_949185
DEFINITION = PREDICTED: similar to MAX-interacting protein isoform 6

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_733751
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 3 isoform 2

ACCESSION = NP_059129
DEFINITION = myosin IIIA

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_612449
DEFINITION = slit-like 2

ACCESSION = XP_935039
DEFINITION = PREDICTED: piccolo isoform 2

ACCESSION = XP_939204
DEFINITION = PREDICTED: piccolo isoform 3

ACCESSION = NP_000495
DEFINITION = coagulation factor X precursor

ACCESSION = NP_036273
DEFINITION = DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26

ACCESSION = NP_067053
DEFINITION = myeloid/lymphoid or mixed-lineage leukemia 3 isoform 1

ACCESSION = XP_931818
DEFINITION = PREDICTED: similar to myoferlin isoform b

ACCESSION = NP_149357
DEFINITION = UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 3

ACCESSION = NP_612446
DEFINITION = G protein-coupled receptor associated sorting protein 2

ACCESSION = NP_005834
DEFINITION = signal transducing adaptor molecule 2

ACCESSION = NP_002580
DEFINITION = protocadherin 7 isoform a precursor

ACCESSION = NP_055495 XP_371036
DEFINITION = hypothetical protein LOC9703

ACCESSION = XP_940803
DEFINITION = PREDICTED: similar to Piccolo protein (Aczonin) isoform 7

ACCESSION = NP_872395
DEFINITION = 5-hydroxytryptamine receptor 3 subunit E

ACCESSION = NP_008965
DEFINITION = DnaJ (Hsp40) homolog, subfamily B, member 4

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****
The pattern being searched is npf.*npf

89 matches found in protein
##############################
ACCESSION = NP_060139
DEFINITION = hypothetical protein LOC54821 isoform a

ACCESSION = NP_438173
DEFINITION = secretory carrier membrane protein 1 isoform 2

ACCESSION = NP_060109
DEFINITION = dachsous 2 isoform 1

ACCESSION = XP_946431
DEFINITION = PREDICTED: similar to Nucleoporin-like protein RIP (HIV-1 Rev-binding protein) (Rev interacting protein) (Rev/Rex activation domain-binding protein) isoform 1

ACCESSION = NP_065855
DEFINITION = protein kinase C and casein kinase substrate in neurons 1

ACCESSION = NP_037465
DEFINITION = epsin 1

ACCESSION = NP_004778
DEFINITION = slit homolog 2

ACCESSION = NP_891555
DEFINITION = fms-related tyrosine kinase 4 isoform 1

ACCESSION = NP_550434
DEFINITION = asialoglycoprotein receptor 2 isoform a

ACCESSION = NP_683723
DEFINITION = epsin 2 isoform a

ACCESSION = NP_003052
DEFINITION = slit homolog 1

ACCESSION = NP_002011
DEFINITION = fms-related tyrosine kinase 4 isoform 2

ACCESSION = NP_001007793
DEFINITION = neurotrophic tyrosine kinase, receptor, type 1 isoform 3

ACCESSION = XP_950234
DEFINITION = PREDICTED: similar to Nucleoporin-like protein RIP (HIV-1 Rev-binding protein) (Rev interacting protein) (Rev/Rex activation domain-binding protein) isoform 3

ACCESSION = NP_060650
DEFINITION = DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 32

ACCESSION = NP_004161
DEFINITION = solute carrier family 1, member 1

ACCESSION = NP_001009954
DEFINITION = hypothetical protein LOC54821 isoform b

ACCESSION = NP_005689
DEFINITION = secretory carrier membrane protein 3 isoform 1

ACCESSION = NP_075049
DEFINITION = B-cell CLL/lymphoma 11B isoform 2

ACCESSION = NP_038472
DEFINITION = ubiquilin 2

ACCESSION = NP_006140
DEFINITION = galectin 4

ACCESSION = XP_086287
DEFINITION = PREDICTED: similar to protein tyrosine phosphatase, receptor type, V

ACCESSION = NP_060759
DEFINITION = VPS53 protein

ACCESSION = NP_001008938
DEFINITION = colonic and hepatic tumor over-expressed protein isoform a

ACCESSION = NP_003585
DEFINITION = transcription termination factor, RNA polymerase II

ACCESSION = NP_006067
DEFINITION = HIV-1 Rev-binding protein-like protein

ACCESSION = NP_001005736
DEFINITION = lysosomal trafficking regulator isoform 2

ACCESSION = NP_115892
DEFINITION = G protein-coupled receptor 145

ACCESSION = NP_001501
DEFINITION = glutamate receptor, ionotropic, delta 2

ACCESSION = NP_009160
DEFINITION = protein kinase C and casein kinase substrate in neurons 2

ACCESSION = NP_689992
DEFINITION = hypothetical protein LOC256710

ACCESSION = XP_946113
DEFINITION = PREDICTED: similar to protein tyrosine phosphatase, receptor type, V

ACCESSION = NP_055621 XP_048462
DEFINITION = RUN and SH3 domain containing 2

ACCESSION = NP_055336
DEFINITION = SH3-domain binding protein 4

ACCESSION = NP_056067
DEFINITION = EH domain binding protein 1

ACCESSION = NP_116561
DEFINITION = synaptotagmin-like 2 isoform a

ACCESSION = NP_851782
DEFINITION = WD repeat domain 17 isoform 2

ACCESSION = NP_061937
DEFINITION = sidekick 2

ACCESSION = NP_877439 XP_294213 XP_353565
DEFINITION = putative binding protein 7a5

ACCESSION = NP_002520 NP_001007205
DEFINITION = neurotrophic tyrosine kinase, receptor, type 1 isoform 2

ACCESSION = NP_597676
DEFINITION = titin isoform novex-1

ACCESSION = XP_947084
DEFINITION = PREDICTED: similar to ataxin-1 ubiquitin-like interacting protein isoform 1

ACCESSION = NP_612808
DEFINITION = B-cell CLL/lymphoma 11B isoform 1

ACCESSION = NP_057340
DEFINITION = N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase precursor

ACCESSION = NP_003053
DEFINITION = slit homolog 3

ACCESSION = NP_009097
DEFINITION = phosphatidylinositol-binding clathrin assembly protein isoform 1

ACCESSION = NP_149095
DEFINITION = stonin 2

ACCESSION = NP_078997
DEFINITION = zinc finger homeodomain 4

ACCESSION = NP_055474
DEFINITION = hypothetical protein LOC9677 isoform 2

ACCESSION = NP_597681
DEFINITION = titin isoform novex-2

ACCESSION = NP_055779
DEFINITION = epsin 2 isoform b

ACCESSION = NP_733828
DEFINITION = WD repeat domain 17 isoform 1

ACCESSION = NP_001012331
DEFINITION = neurotrophic tyrosine kinase, receptor, type 1 isoform 1

ACCESSION = NP_443069
DEFINITION = secretory carrier membrane protein 3 isoform 2

ACCESSION = NP_060427
DEFINITION = epsin 3

ACCESSION = NP_005688
DEFINITION = secretory carrier membrane protein 2

ACCESSION = NP_001032
DEFINITION = sucrase-isomaltase (alpha-glucosidase)

ACCESSION = NP_055571
DEFINITION = colonic and hepatic tumor over-expressed protein isoform b

ACCESSION = NP_079199
DEFINITION = nucleoporin 210

ACCESSION = NP_003310
DEFINITION = titin isoform N2-B

ACCESSION = NP_003886
DEFINITION = synaptojanin 1 isoform a

ACCESSION = XP_950699
DEFINITION = PREDICTED: similar to ataxin-1 ubiquitin-like interacting protein isoform 2

ACCESSION = NP_003256
DEFINITION = toll-like receptor 3

ACCESSION = NP_001334
DEFINITION = disabled homolog 2

ACCESSION = NP_596869
DEFINITION = titin isoform N2-A

ACCESSION = NP_055719
DEFINITION = RAB11 family interacting protein 2 (class I)

ACCESSION = NP_001005236
DEFINITION = olfactory receptor, family 1, subfamily L, member 1

ACCESSION = NP_055061
DEFINITION = cadherin EGF LAG seven-pass G-type receptor 1

ACCESSION = NP_203744
DEFINITION = molecule interacting with Rab13

ACCESSION = NP_004495
DEFINITION = HIV-1 Rev binding protein

ACCESSION = NP_000072
DEFINITION = lysosomal trafficking regulator isoform 1

ACCESSION = NP_001009944
DEFINITION = polycystin 1 isoform 1 precursor

ACCESSION = NP_085058 NP_078949 XP_290704
DEFINITION = WD repeat domain 59

ACCESSION = NP_444295
DEFINITION = ubiquilin 1 isoform 2

ACCESSION = NP_056040 XP_041018

DEFINITION = BNIP2 motif containing molecule at the carboxyl terminal region 1

ACCESSION = NP_036273
DEFINITION = DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26

ACCESSION = NP_001005234
DEFINITION = olfactory receptor, family 1, subfamily L, member 3

ACCESSION = NP_006251
DEFINITION = DnaJ (Hsp40) homolog, subfamily C, member 3

ACCESSION = XP_943327
DEFINITION = PREDICTED: similar to Zinc finger protein 486

ACCESSION = NP_060250 XP_098762
DEFINITION = chromodomain helicase DNA binding protein 7

ACCESSION = NP_071735
DEFINITION = FYVE-finger-containing Rab5 effector protein rabenosyn-5

ACCESSION = NP_000287
DEFINITION = polycystin 1 isoform 2 precursor

ACCESSION = NP_038466
DEFINITION = ubiquilin 1 isoform 1

ACCESSION = NP_004857
DEFINITION = secretory carrier membrane protein 1 isoform 1

ACCESSION = NP_064516
DEFINITION = ataxin-1 ubiquitin-like interacting protein

ACCESSION = NP_038478
DEFINITION = bromodomain adjacent to zinc finger domain, 2B

ACCESSION = NP_001172
DEFINITION = asialoglycoprotein receptor 2 isoform a

ACCESSION = XP_950232
DEFINITION = PREDICTED: similar to Nucleoporin-like protein RIP (HIV-1 Rev-binding protein) (Rev interacting protein) (Rev/Rex activation domain-binding protein) isoform 2

ACCESSION = NP_002368
DEFINITION = MAS1 oncogene

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